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INVESTIGATIONS ON THE ABSORPTION OF AEROSOLS IN HUMANS

NATURAL FUNCTION OF THE MUCOSA OF THE RESPIRATORY PASSAGES AND THEIR ALTERATION BY ADMIXTURES IN THE AIR

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Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; pp 16-21

The mucus membrane of the respiratory passages is a sheet-like organ whose typical parenchyma, the multi-layer ciliated epithelium and the mixed glands cover and/or sink into a regional, partly differentially composed mesenchymal basic substance. In its entirety the respiratory mucus membrane is a boundary layer of the body which on one hand prepares the air for the gas exchange in the alveoli by warming, humidifying and purifying the air and regulating the air current, and on the other hand the rapidly and abundantly reacting boundary layer has the task of adapting the organism to its gaseous environment and the impurities in the latter. We shall discuss here, mainly, these latter functions.

Let us attempt to first follow purely theoretically the processes which are responsible for secretion and resorption as the most striking life phenomena of the mucus membrane. The blood circulation carries, by means of its hydrostatic pressure, by the oncotic properties of the proteins and the characteristics of the capillary membrane, food- and secretion-building substances into the glandular and ciliated cells through the intermediary of the connective tissue, and carries away metabolic and resorption products partly over the venous capillary system and partly over the lymphatic vessels. From the substances supplied to it the gland cell synthetizes the specific secretion-building substance which represents at least to a great extent the dispersed phase of

the secretion. The secretion granulum is to be found in the gland cells in a form of gel and will be solvated at the time of the emit from the cell. Where the origin of the dispersing medium is, in the gland cells themselves or perhaps in the epithelium of the excretory duct, is not yet fully clarified; the latter, however, is possible, since the excretory ducts are surrounded by such a dense capillary network that at one time the latter was considered to be an erectile body that regulates the secretion in a purely mechanical manner. Through the solvation in the excretory duct a considerable increase in volume is attained, and in this way the secretion is urged toward the surface. This solution pressure -- the oncotic pressure -- of the secretion regulates the giving off of moisture to the secretion surface and at the same time may supply moisture from the depths through the action of liberated forces. The speed of the processes linked with the oncotic and osmotic pressure of the secretion is actively supported by turbulent-flow phenomena which occur in the flow of secretion due to various factors. From the secretion of the surface substances are constantly taken up by the mucosa so that in a certain sense there is maintained a circulation of secretion from the gland through the surface secretion and back again to the mucosa. When one combines these processes occurring in the secretion and resorption of the respiratory mucosa, it is seen that there are three circulations superposed on one another, namely, the blood circulation, the "inner circulation," and a kind of secretion circulation. In pathological events this has the consequence that a dysfunction of only a single quality must involve the other, superimposed, functions in the pathology.

Let us now turn to the mucus membrane functions which are of particular interest for our subject:

The cilia of the ciliated epithelium carry out 160-250 beats per minute, or 2 to 12 per second, at a temperature optimum of 30°, whereby a beat lasts between 1/4 and 1/10 of a second. A differentiation is made between an activity and a recovery beat; the former is faster and stronger, lasts about 1/3 of the beat cycle and is decisive for the direction of the transport of the secretion cover. The frequency and amplitude of the ciliary beat may vary, while the rhythm and form remain essentially constant. Decisive for the transport performance is the amplitude. Acceleration over a maximal velocity takes place at the expense of the amplitude and therefore of the work performance. The ciliated epithelium of the respiratory mucosa develops per cm^2 and per minute a lifting force of 7 g; the performance can

then be increased for a short time after which, in any event, fatigue phenomena will set in. In the region of the excretory ducts of the secondary cavities the performance is several times greater. The working power of the ciliated epithelium depends, among other things, on the temperature, the pH of the tissue and secretion, the viscosity and surface tension of the secretion, etc. The temperature optimum lies between 18 and 33°C. Cold is not a damaging factor; an increase of temperature above 35° leads to an increase of frequency and a rapid decrease of working power until the flow of secretion ceases, and at 43-44°C the cilia irreversibly stop functioning. A shift of pH to above 8.0 or below 6.4 causes a cessation of the ciliary movement. Drying of the secretion surface causes an increasing slowing-down and dryness, and after 15 minutes or longer an irreversible stoppage occurs.

In the epithelia not only the cilia of individual cells undertake successive beats but also the cilia of the same cell display a serial sequence in their movement. Through these beats following one another there is created a longitudinal wave motion which progresses in the direction of the recovery beat. The beats are directed, in the nose, backwards and downwards, in the secondary cavities toward the ostia, and in the tracheobronchial tree also toward the pharynx.

The secretion from the nose normally enters the epipharynx at the edge of the choana and flows mainly from the tube opening over the side cord in the pharynx. In the epipharynx a second secretion district makes its start, originating from the glands of the roof of the thorax, the mixed glands of the pharyngeal velum and the glands of the side cord. In the tracheobronchial space the secretion is moved in the direction of the larynx. Hence t does not move over the shortest route but spirals upwards in a clockwise direction. The combs of the bronchial partition sites are thus avoided and circumvented on both sides. Hence the flow velocity is independent of the position of the subject, so that apparently the force of gravity is less important than the viscosity and surface tension of the secretion and the beating force of the cilia. The flow velocity in the nose is taken on the average as 0.25 to 0.75 cm/min. In the posterior two thirds of the nose it may attain 1.0 cm. Accordingly the entire secretion coating of the nose should be renewed every 30-45 minutes at the latest. The trachea may pass the secretion through in 30 minutes, whereby a velocity of 0.8 to 3.5 cm/min has been measured. The stream of secretion is not influenced by small injuries such as, for instance,

small cuts, since the viscosity of the secretion bridges over such small defects; it slides around small epithelial faults. Small individual ulcers placed by means of a thermocauterizer circularly into the trachea in such a way that there is always a strip of undamaged epithelia between them bring about a full cessation of the flow of secretion. At places of mucosa of the tracheobronchial tree that carry no ciliary epithelium and which may occur normally in the trachea in the region of the membranous wall the secretion may under certain circumstances remain stationary for a longer period of time.

From the secretion or through its intermediary a large number of substances is absorbed by the mucosa. For gases this is best demonstrated by the prompt initiation of action after inhalation of amyl nitrite. During its flow through the nose the respired air loses 0.1% of its oxygen while the CO_2 content rises by the same amount. In a closed side cavity in 3 1/2 hours there is a drop in the concentration of the oxygen of air from 21 to 9% and there is also a pressure drop which, however, may be equalized depending on the secretion conditions and the giving off of CO_2. The uptake of solutions of the most varied type has been repeatedly tested through the application of medicines and dye solutions; in this connection some substances are absorbed so well and quickly that they act right after a subcutaneous injection (for example hyoscine), while others are poorly resorbed. Comparative investigations of mucosa of different organs revealed in every case that the resorption of the mucosa of the respiratory tract, where it is covered with ciliated epithelium, is hardly worse than that of the intestine. As for the colloids among which the proteins, above all, were investigated, it was found that they can pass through the mucosa up to a molecular weight of 70,000 -- which corresponds to the albumin, while larger molecules will not be absorbed. Since, however, one can cause an anaphylactic shock through inhalation in animals intraperitonally sensitized with horse serum, it appears that also horse serum or parts thereof may be taken up by the mucosa or that the former enters into the most intimate contact with the mucosa. -- As regards solid substances, the general view is that, to the extent that they are soluble, they are dissolved in the secretion and then may be resorbed (for instance, treatment of diabetes insipidus with hypophyseal anterior lobe snuff powder, among other things). Insoluble particles over 1 μ in size are supposedly incapable of passing through the intact nasal mucosa; in some cases, however, this does seem possible. -- The uptake of pollen up to a size of 30 μ in sensitized and non-sensitized

mucosa could be histologically followed, and it was found that pollen could pass through the sensitized mucosa in 5-10 minutes and in great numbers. (A thorough demonstration of the resorption of various substances is given by H. H. Naumann in his film: "Intravital Observations in the Nasal Mucosa.")

The state and reaction condition of the total organism widely affect all mucous functions and determine the type and extent of their response to various influences. How quickly, for example, the ciliated epithelium responds to the stimulation of the vegetative system may be seen from the following observation: in the guinea-pig trachea the epithelial cells are the same in shape and relations in various bounded sections. The individual parts bordering on one another, however, show distinct differences; thus secreting, emptying and filling up take place in different functional periods. Now when the sympathetic or parasympathetic system is stimulated by ephedrin resp. pilocarpine, there occurs a rapid emptying of the epithelial section ready for secretion, and also typical changes in the region of the ciliary cells which the sections in other function phases quickly follow. Forty minutes after a parasympathetic stimulus there begins in the basal nuclear series an intraepithelial gland formation through the formation of mucus in the individual neighboring cells; this gland formation reaches its peak already 20 minutes later, that is, 60 minutes after the application of the stimulus, and may again disappear 30 minutes thereafter. Stimulation of the sympathetic or parasympathetic system induces a function cycle in the epithelium which again attains the initial phase after 60-90 minutes. Despite the strongest stimulation there remains here a subdivision, in epithelial sections, into different more or less intact functional stages. After a single atropine injection one finds, as the maximum of alterations after 90 minutes, the nuclei greatly enlarged in the large epithelial areas, with clear nuclear membrane and a sharply colored recticulum, while the nuclear lumen appears empty. The protoplasm in these cells is mostly pushed to the edge and is of a finely reticulated structure. These cell pictures may come about, among other ways, through an increased sympathetic influence, since the pictures obtained after administration of an overdose of thyroxine show some similarity with them. Weeks-long administration of ganglion-blocking media cause no histologically detectable epithelial changes. It seems, however, that under these conditions the epithelium is more resistant to the effect of different intoxications, an observation which could perhaps indicate that in the development of pathological

epithelial changes pathological tone changes of the vegetative system play an essential role in some way.

These examples of the dependence of the type and intensity of reaction of the respiratory mucosa on internal influences may be multiplied at random: for instance a single severe parenteral intoxication with allyl formate leads to epithelial lesions which correspond to an acute inflammation; a chronic intoxication with the same poison, however, causes an epithelial hyperplasia. These observations are brought up only in order to show to what extent the response of the mucosa to influences of air impurities is dependent on the general condition of the organism and the conditions which it bids to the mucous organ.

But how does the mucosa behave toward air impurities? Let us only mention a few examples:

Bacteria which get on the nasal mucosa will under normal circumstances be held by the secretion and to a great extent carried away by the latter; some bacteria are destroyed by the secretion already after 5-10 minutes. Under experimental conditions it was found that virulent bacteria and their toxins can be quickly absorbed; thus, for instance, virulent pneumococci placed on the nasal mucosa of mice can be cultured from their cardiac blood already 10 minutes later. It was noted in histological sections that apathogenic staphylococci, hay bacilli and sarcines were present in the epithelial cells already 15 minutes after their introduction; they were "phagocyted" 1 hour later by small round cells, and 6 hours later they had disappeared from the mucosa. By contrast anthrax bacilli penetrated between the epithelial cells and had imigrated into the submucosa already after 15 minutes; they had likewise disappeared from the mucosa after 6 hours. Some authors represent the view that apathogenic bacteria are actively taken up by the epithelial cells of the respiratory pathways. These observations illustrate the task of the mucosa of adapting the organism to the environmental conditions which affect it in the gaseous state, since the bacteria cause the formation of antibodies through the respiratory mucosa. This is an ability which has been and is repeatedly and practically utilized; already the ancient Chinese knew that they could confer immunity against cow pox through the introduction of dried cow-pox crusts.

How does the respiratory mucosa behave toward proteins? In addition to current experiences from daily practice it is known from experiments that one may bring about allergic

phenomena resp. an anaphylactic shock through the inhalation of antigen after parenteral and local sensitization. In this connection I find very interesting the observations which I could make on animals which had repeatedly inhaled their own blood plasma: the histological changes were the same as occurred after foreign plasma, and were typical allergic changes, only they were milder in their development and required a longer time to set in. Similarly interesting are the observations which I made on adrenectomized guinea pigs. The removal of both adrenals leads to no detectable histological changes in the respiratory mucosa. When, however, such animals obtained small amounts of substituted Doca and then made to inhale, for a single time, foreign plasma, all animals displayed massive alterations between the subepithelial elastic fiber reticulum and the epithelial base, thus in the region of the basal membrane; in a short time there were formed there either massive hyaline intercalations or collections of lamellar connective tissue which corresponded to the changes of a papillary hyperplasia.

Were we to draw the conclusions from these observations, we come to the following: the function of the mucosa of the respiratory passages is, in addition to preparing the air for the gas exchange, to adapt the organism to its gaseous surroundings; further: the alteration of the mucosa by air admixtures resp. the response of the mucosa thereto is highly dependent on the mometary reaction condition of the organism and its particular characteristics, for example, in regard to the hormone economy, metabolism, the vegetative situation, etc.

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BRONCHOSCOPIC OBSERVATIONS AFTER INHALATION OF DYES UNDER NORMAL AND PATHOLOGICAL CONDITIONS IN THE RESPIRATORY TRACT

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Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; pp 22-51

The first reports on bronchoscopic investigations following inhalation of dyes in human subjects were published at the start of this century by von Schrotter (1904) and Safrack (1911). They did not, however, obtain any evaluable results since the tube which had been introduced deeply produced coughing and the sparsely colored secretion was immediately thrown out. On the basis of additionally performed animal experiments the two authors merely reported that there occurs a blue coloration at the point of partition of the tracheobronchial tree, thus at the point where the air current encounters a resistance and not in the tube sections lying in between; only in the case of abundantly occurring mucus was any coloration observed also at the latter site.

More exact presentations regarding the flow of inhaled liquid droplets in the respiratory passages originate from Heubner (1920). He found with the glass anatomical model that fine mists flow through straight passages without losses, even when the latter are constricted or divided. Only in the case of narrow tubes do curvatures cause an increase in the deposition of particles. The most intense precipitation takes place on widening of a narrow flow path, even when its course is straight. Transferred to the conditions in the human respiratory tract, Heubner stated the following: "One may declare that the probability is quite high that the main portion of an inhaled mist penetrates quite deeply into the bronchial tree without any considerable loss, and that on

inspiration some precipitate will adhere only at larger curvatures. Above all, however, at the moment of the reversal of the direction of the respiratory air much of the inhaled mist will reach the wall of the bronchi due to vortex movements which have set in. During the expiratory phase additional amounts of precipitate will settle at all branching sites, particularly at the meeting of small and much larger bronchi. The characteristic color of the tongues between two bronchial openings originates in my opinion above all from the vortex formation during expiration. From another angle the stronger coloration of the trachea would at least to some extent be due to the vortex formation on the side of the glottis during inhalation; it is probable that the absolute size of the cleft of the glottis is of significance for the amount of precipitate forming there."

Since then bronchoscopy has become a routine clinical investigational method; and since in addition there is the possibility of fixing the observed findings photographically, our investigation is no longer faced with any particular difficulties. Before, however, we report on our findings, we wish to make, for the sake of better understanding, some clarifications regarding the anatomy and physiology of the tracheobronchial tree.

Fig. 1 and 2 show the external view of the tracheobronchial tree from the front and back, respectively, up to the division into sub-branches. One sees in the region of the air tubes in both main- and upper-bronchial lobes, in regular arrangement, transverse cartilaginous spangles. The latter are separated in the front by means of short connective-tissue septa, while the ends of the cartilages are connected in the back by a transversal, piercing membrane, the so-called paries and membraneaceus. The tube is elastic and may be extended or constricted. As it may further be seen from Figs. 1 and 2, the cartilage framework is no longer regularly arranged in the region of the top lobe, middle lobe and segmental bronchi; here cartilaginous plates are distributed in all wall sections. In addition it must be noted that the direction in which the right main bronchus extends is almost the same as that of the trachea, while the left main bronchus deviates more toward the side. The right top-lobe bronchus originates from the main bronchus already after 2 cm and takes its course sideways almost at a right angle. On the other side, the departure of the left top-lobe bronchus occurs very much deeper (5 cm) and likewise tends in a lateral direction. It is divided after a short distance into a branch tending laterally, frontally and downwards (lingual

bronchus), and one tending laterally, upwards and backwards (bronchus of the rising segment group). Also, these bronchi divide quickly into further branches, the so-called segmental bronchi, which now proceed in all directions and are again divided into so-called sub-segmental bronchi. The branching of the lobe bronchi into segmental and subsegmental bronchi may be seen well in Figs. 1 and 2, in which the numbers denote the segmental bronchi (10 on the right and 9 on the left).

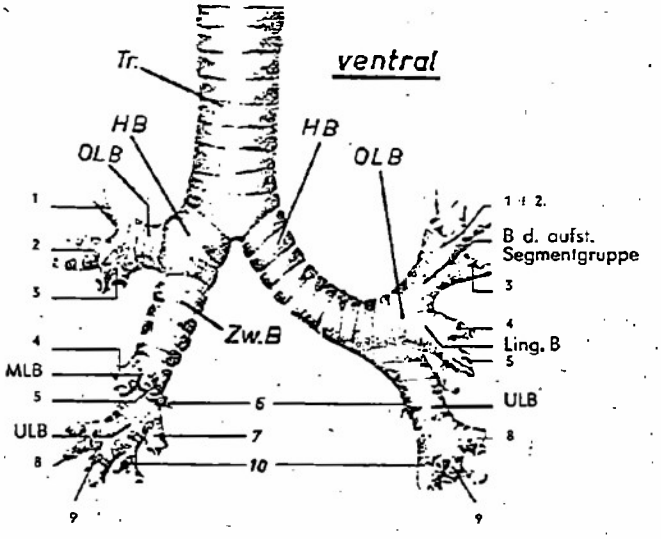

Fig. 1. Ventral View of Tracheobronchial Tree Up to the Subsegment Bronchi. For explanation of symbols, see Table 1 and 2, as well as text. [B d. auft. Segmentgruppe = Bronchus of the ascending segment group].

Table I lists the names of segmental bronchi, separately for the right and left sides of the bronchial tree, according to the international London nomenclature (1949).

In addition, we have summarized, in Table 2, the abbreviations customary in bronchology. They are used in all figures. Thus, for example, LHB = left main bronchus; ROLB = right top-lobe bronchus; SB 3 = anterior segmental bronchus, etc.

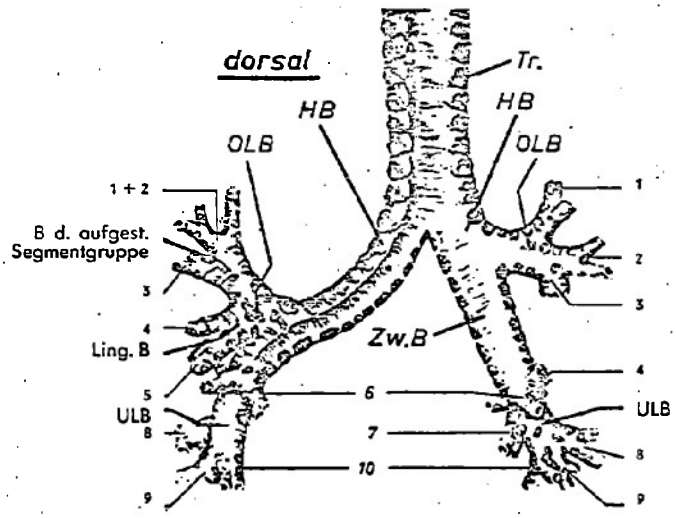

Fig. 2. Dorsal View of the Tracheobronchial Tree.

TABLE 1

Designation of the Segmental Bronchi of the Right and Left Bronchial Tree According to the London Nomenclature (1949)

1 -- Right Bronchial Tree; 2 -- Lobe; 3 -- Left Bronchial Tree; 4 -- Top Lobe; 5 -- Middle Lobe; 6 -- Bottom Lobe; [SB = Segmental Bronchus].

The reason for our having so thoroughly reproduced the bronchial anatomy all the way to the segmental bronchi is that with the help of modern wide-angle lenses it is

possible to view directly the mucosa up to these regions. Further, anatomical (Aeby, Ewart, Herrnheiser and Kubat, Boyden, V. Hayek), bronchoscopic (Kramer and Glass, Jackson, Soulas and Mounier-Kuhn, Lemoine), bronchographic (Huizinga, Stutz, Esser) and surgical (Brock, Forster-Carter, Overholt) studies have shown that the segments are constantly occurring lobe-parts, and their bronchi have a quite definite direction of course. During the last two decades these findings have turned out to be very fruitful in the diagnosis, pathology and therapy of lung diseases.

TABLE 2

Usual Abbreviations for Sections of Tracheobronchial Tree
[To be used as Key to Figs. 1 and 2]

Trachea (Tr)	
Intermediate Bronchus (ZwB)	
Main Bronchus (HB)	Bronchus 1. Class
Top-Lobe Bronchus (OLB)	
Middle-Lobe Bronchus (MLB)	
Lingula Bronchus (Ling.B.)	Bronchus 2. Class
Bottom-Lobe Bronchus (ULB)	
Bottom-Lobe Bave Bronchus (ULBB)	
Segmental Bronchus (SB, No 1-10)	Bronchus 3. Class
Subsegmental Bronchus (SSB)	Bronchus 4. Class

What the inside of the trachecbronchial tree looks like is represented, semi-schematically, in Fig. 3, and in addition it can be seen from original photographs. In correspondence with the investigational position during the bronchoscopic examination, the bronchial tree in Fig. 3 is turned 180° in relationship to Figs. 1 and 2. The circles within the air tubes and bronchi indicate the typical branching sites of the bronchial tree. Accordingly one sees in the lowest ring the partition of the trachea into the two main bronchi (bifurcatio tracheae) with the projecting ridge in the middle (carina tracheae). Then to the right is drawn the division of the main bronchus into the middle bronchus/top-lobe bronchus, and to the side thereof the division of the top-lobe bronchus into the three segmental bronchi, below this the division of middle-lobe bronchus/bottom-lobe bronchus, and finally the division into the bottom-lobe segmental bronchi.

On the left we first see the division main bronchus/upper-lobe bronchus, then the division of the upper-lobe

bronchus into the individual branches, and on the deepest level the division of the bottom-lobe bronchus into the segmental bronchi.

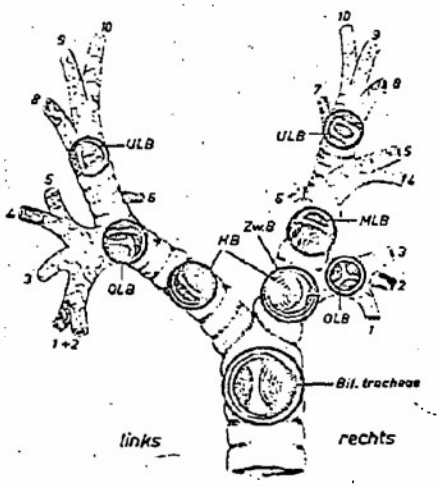

Fig. 3. Semi-Schematic Bronchoscopic View of Various Sections of the Tracheobronchial Tree. [Links = left; rechts = right; Bif. tracheae = bifurcatio tracheae].

For the sake of completeness we will mention here that the inside of the tracheobronchial tree is covered with a mucus membrane. The latter is normally covered with a thin, transparent mucus layer into which project processes of the ciliated epithelium. It was found in animal experiments that through thythmic movements of the cilia corpuscular elements are carried upwards with a velocity of 1-2 cm/min. The ciliary activity is nevertheless very sensitive to, and affected by, many external and internal influences (Antweiler).

Up to now we have considered the tracheobronchial tree as an isolated structure. In reality it is expanded with the lung parenchyma, the lung vessels and the pleura in the thorax. In order to obtain an idea regarding the spacial relationships, we have represented the course of the tracheobronchial tree, on the basis of bronchographic investigations, up to the segmental bronchi in relation to the pulmonary lobes (solid line) and to the segments (broken line). Fig. 4 shows the extension of the tracheobronchial

tree into the lung in front view and in right and left side views; wherein the vertebral column and the sternum are drawn in an additional point of reference. It can be seen that the bronchi become even smaller toward the periphery and that, with the exception of the right upper-lobe bronchus, they always form two branches (dichotomy). It is noteworthy that, as was already mentioned, the bronchial course is always constant up to the region of the segmental branches, so that all sections occupy a quite definite position in the thoracic space. From the subsegmental bronchi on toward the periphery the variability of the bronchial branches increases to such an extent that it is no longer possible to obtain an accurate anatomical identification with the currently available clinical study methods.

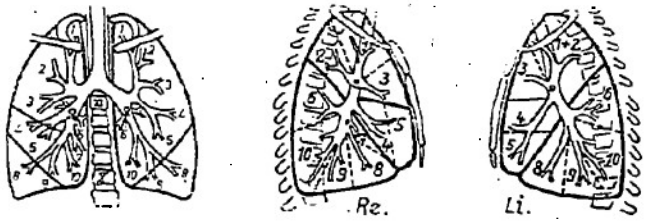

Fig. 4. Scheme of the Tracheobronchial Tree From Front and Side on the Basis of Bronchoscopic Findings. [Re = right; Li = left]

Fig. 5 shows once more, in a schematic manner, the division of the tracheobronchial tree up to the end branches, the alveolar sacs. On the right side of the picture are indicated the individual bronchus sections, on the left, for the sake of completeness, the parenchyma units belonging to the bronchi. The dichotomous branching of the bronchial tree is clearly shown on Fig. 5, though in reality the two branches are of differing sizes. There is a rule that the branch having the narrower lumen deviates more from the original direction than the one having the wider lumen. Hence, apart from the large bronchi there does not exist a direct route for the air current. Also, despite the constant decrease of the individual bronchial cross sections the total diameter constantly increases. For this reason, too, the air flows toward the depth unimpeded. On the other hand this results in a decrease of the flow velocity. Due to the nearly ideal construction of the tracheobronchial tree under normal conditions streamlined flow predominates. Only at very high air velocities, such as occur on forced expiration and a cough spasm, does there take place any vortex

formation (Rohrer). The development of vortex formation must also be discussed in connection with abrupt cross-sectional and directional changes and at branching sites when the flow takes place around sharp edges. The physical bases of the movement of individual aerosol particles, the properties of clouds of particles and the behavior of the aerosol stream in the respiratory passages have been recently summarized by Dirnagl. For the sake of completeness it must be added that Findeisen, in his work which has since become classic (1935) has determined the degree of separation of various article sizes in the respiratory passages on the basis of physical and mathematical considerations. Anacker has added to the latter study data regarding settling in the segmental bronchi (Table 3).

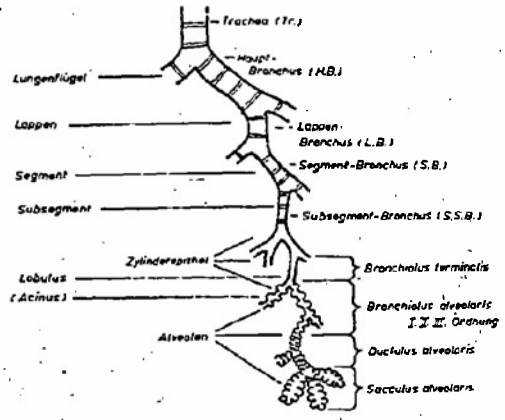

Fig. 5. Schematic Diagram of the Tracheobronchial Tree Up to the End Sections. [Lungenflugel = lobe of the lungs; Lappen = lobe; Zylinderepithel = cylinder epithelium; Alveolen = alveoli; Hauptbronchus = Main Bronchus; Lappenbronchus = lobe bronchus; Ordnung = Class].

In Table 4 are given the diameter, length and angle of flow change in the individual sections of the tracheobronchial tree up to the segmental bronchi, separately for right and left side. They are the values of Anacher, Brunings, Parchet and Stutz, which are summarized on the basis of investigations on cadaver lungs, X-ray pictures and bronchographic and bronchoscopic measurements. The range of fluctuation determined by age, sex and constitution, is quite considerable. In addition we have entered in the table the flow velocity according to Findeisen.

TABLE 3

Calculated Values for the Settling of Aerosols of Varying Particle Size in the Tracheobronchial System (Up to the Segmental Bronchi) in Percent of the Amount of Particles Present in the Trachea on Entry (Anacker, in Appendix to Findeisen)

Bronchialabschnitt	Teilchengröße r = 1 μ		r = 2 μ		r = 10 μ		r = 20 μ	
	rechts	links	rechts	links	rechts	links	rechts	links
Trachea	0,1		0,36		7,8		26,0	
Bifurc. HB	0,057	0,078	0,227	0,312	5,25	7,2	19,2	26,4
HB	0,11	0,11	0,358	0,358	6,6	6,46	14,2	12,2
Car. HB/OLB	0,19	0,14	0,765	0,566	15,6	11,25	<40,6	<35,2
OLB	0,12	0,12	0,197	0,197	2,9	1,47	Ø	Ø
Car. OLB/SB_1	0,082—0,236	0,12	0,322—0,92	0,47	5,1—14,6	8,0	Ø	Ø
Car. OLB/SB_2	0,082—0,216	bis 0,226	0,32 —0,85	bis0,89	5,1—13,4	bis 14,9	Ø	Ø
Car. OLB/SB_3		0,12	0,61	0,47	9,6	8,0	Ø	Ø
ZwB/MLB	0,082	0,101	0,322	0,393	6,0	6,6	Ø	Ø
ZwB	0,064	0,134	0,254	0,525	5,38	10,5	15,4	34,7
ULB	0,12	0,12	0,198	0,197	2,9	1,47	13,1	<0,5
ULB/SB_6	0,23	0,25	0,935	0,982	17,2	16,8	<12,1	Ø
ULBB/SB_7	0,22	—	0,875	—	16,2	—	Ø	Ø
ULBB/SB_8	0,14	0,14	0,576	0,565	10,5	9,6	Ø	Ø
ULBB/SB_9	0,12	0,14	0,485	0,565	9,0	9,6	Ø	Ø
ULBB/SB_{10}	bis 0,007	bis 0,009	bis 0,296	bis0,39	bis 0,5	bis 0,6	Ø	Ø

[1 -- Particle Size; 2 -- Bronchial Section; 3 -- right; 4 -- left; Car. = Carina; bis = to; for rest of abbreviations, see Table 2.]

Material and Method

The investigations were carried out on 22 patients in whom a bronchoscopy was indicated for diagnostic reasons. The ages varied between 19 and 65 years. As is shown in Table 5, in addition to normal cases we used also patients with pathological changes in the bronchial system, lungs parenchyma, pleura and those after collapse operations. For the sake of completeness other pathological were also entered, states in which one expected certain peculiarities in distribution of the inhaled dye.

TABLE 4

Diameter, Length, Flow-Path Change Angle and Flow Velocity in Different Sections of the Tracheobronchial Tree up to the Segmental Bronchi

	Durchmesser in mm		Längenmaße in mm		Strombahn-änderungs-winkel ∢		Strömungs-geschwindig-keit in cm/sec	
Trachea	13—22		100—120				150	
Bronchialbaum:	rechts	links	rechts	links	rechts	links	rechts	links
HB	12—16	10—14	10—25	45—50	20°—30°	25°—45°	150	150
OLB	8—9	9—10	15	10—20	50°—60°	30°—40°	130	130
SB_1	3,5	5	2—8	5—15	20°—70°	30°—70°	65	65
SB_2	3,5		2—8		20°—65°		65	65
SB_3	3,5	4	3—8	3—10	40°	30°	65	65
ZwB	12—13		20—30				150	
MLB bzw. Ling.-B	6—8	7—8	5—10	5—15	20°	25°	130	130
SB_4	3,0	3,5	2—5	3—4	0°—10°	10°—20°	65	65
SB_5	3,0	3,5	2—6	5—10	10°—20°	0°—10°	65	65
ULB	9—12	10	3—5	3—5	15°	25°	130	130
SB_6	5,0	5,0	2—5	2—5	55°	60°	65	65
ULBB	6—8	4—7	15—20	15—20			130	130
SB_7	3,0		5—10		50°		65	
SB_8	3,5	3,5	2—5	2—5	30°	30°	65	65
SB_9	3,5	3,5	2—7	2—7	25°	30°	65	65
SB_{10}	3,5	3,5	2—8	2—8	0°—15°	0°—20°	65	65

[1 -- Diameter; 2 -- Length; 3 -- Flow-Path Change Angle; 4 -- Flow Velocity in cm/sec; Bronchialbaum = bronchial tree; rechts = right; links = left; bzw. = and/or; for the rest of abbreviations, see Table 2.]

For inhalate a 5% solution of the medical, colloidal, fat-insoluble dye Evans Blue or Geigy Blue (Molecular Weight: 960.8) was administered. Previous inhalation studies with guinea pigs with the same dye, though in a higher concentration (7.5%) and during 30 minutes (Schiessle) had displayed no striking toxicity signs against a particularly nice color contrast on the background of the bronchial mucus membrane. The inhalation time in the present studies varies, in the beginning, between 2 and 5 minutes; later it was always 5 minutes. Side reactions of a serious nature were never observed. Only patients with bronchitis sometimes exhibited minimal excitation phenomena varying from short throat

clearings to, at the very most, light cough spasms. These persons then had to be excluded from the evaluation of the experimental results, since even the slightest coughing spasms carried colored secretion from the periphery to the centrally located bronchi whereby the peculiar inhalation picture is blurred.

TABLE 5

Number of Cases Investigated, Divided According to Illness Groups

Illness Group	Number
Normal Bronchial Tree and normal Lung Parenchyma	2
Bronchitis with or without Hypersecretion with normal Lung Parenchyma	0
Bronchiectases with Lung Shrinkage	1
Bronchus Stenoses of different Degrees with or without Parenchyma Shrinkage	6
Total Stenosis of a Main-, Lobe- or Segmental Bronchus	2
Infiltration of the Parenchyma with open Bronchus	2
Monolateral Lung Shrinkage with or without Caverns with Contortion of the Tracheobronchial Tree	2
Bilateral Lung Shrinkage with or without Caverns with Contortion of the Tracheobronchial Tree	5
Pleural Effusion	1
State after Collapse Operation	1
State after Resection	0
Chest Deformation	0
Disease of the Diaphragm	0
Total	22

Premedication for the bronchoscopy was carried out with Luminal, Megaphen-Atosil, atropine and Dicodid. For local anesthesia Novesin "Wander" was employed. The dye inhalation proper was carried out in two ways. In the first method which we tried first, the dye was inhaled through a tube placed in the trachea, the main-or intermediate-bronchus. A commercial device from the firm Drager (penicillin atomizer, average particle size 2 μ) served as the aerosol source. By means of a specially constructed intermediate piece, shown in Fig. 6 in its assembled and dismantled states, it was possible to leave the inhalation tube permanently in

the bronchoscope. Since, however, the patients breathed relatively abundant amounts of secondary air (Nebenluft), the visible dye precipitate on the bronchi was slight. Therefore we soon adopted a second method which brought better results. Here the patient inhaled the dye in a sitting position, through the mouthpiece, directly after local anesthesia, for five minutes. By means of a nasal clamp nasal breathing was excluded. The depth and frequency of breathing varied according to the extent of the pathological alterations present, the age, etc. However, they were kept constant in each case as much as possible. Immediately after the inhalation, for maximal relaxation Hexamide "Heyden" was additionally injected intravenously on the bronchoscopy table, sometimes causing somnolence. The time between end of inhalation and beginning of the bronchoscopy was 3-5 minutes, never longer.

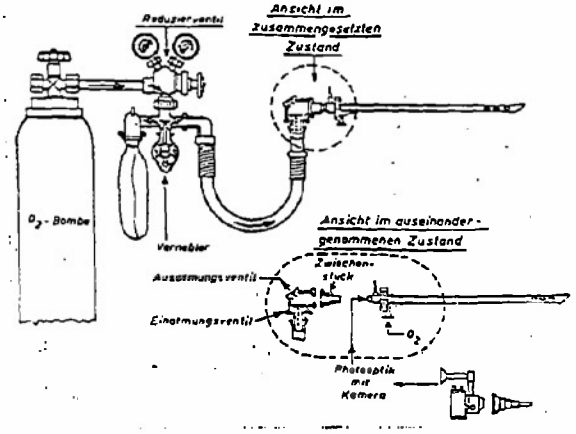

Fig. 6. Sketch of the Experimental Setup with Horizontal Bronchoscope. View of the Intermediate Piece in Dissembled and Assembled State. [Reduzierventil = reducing valve; Ansicht in zusammengesetzten Zustand = View in Assembled State; O_2-Bombe = oxygen bomb; Vernebler = Atomizer; Ansicht im auseinandergeommenen Zustand = View in Dissembled State; Ausatmungsventil = expiration valve; Einatmungsventil = inhalation valve; Zwischenstuck = intermediate piece; Photooptik mit Kamera = Lens with Camera.]

The bronchoscopy was performed in the usual manner. After observation of the trachea we viewed first the right and then the left bronchial tree by means of various

lenses. At places where dye was visible photographs were taken with a device of the Firm Storz, Tuttlingen.

Out of the abundant material available we will describe in the next section a typical normal case and then a few pictures observed in the respiratory tract under pathological conditions. The pictures are black and white reproductions of original color photographs. Only a few places which have lost detail on copying have been retouched.

Results and Discussion

Normal Case: (Figs. 7-13): S. D., 22 years old, male.

5 minute inhalation of 5% Evans Blue prior to bronchoscopy. Amount of air breathed per minute 10 liters; respiratory rate 10/min; dye supported without irritation.

Case 1, Figs. 7-13.

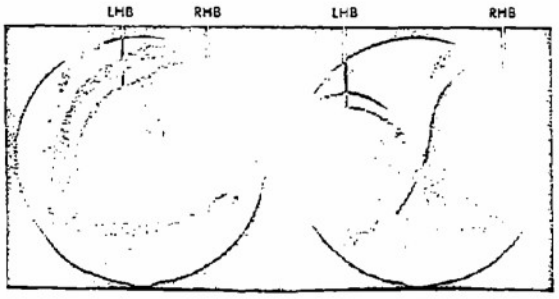

Fig. 7. View From the Lower Air Tube to the Partition Point (Bifurcation) of Same and the Beginning Portion of the Two Main Bronchi (MB). Right picture closeup of the left picture. Dye only visible on carina of bifurcation.

In the region of the upper and middle trachea no coloration visible. On the other hand there is a narrow, streak-like covering on the bifurcation, that is, on the carina (Fig. 7). The wall sections are free, also in the region of the main bronchi (Fig. 8). One notes a dye coating again on the carina, top-lobe/intermediate bronchus and top/bottom-lobe bronchus, left. After pushing the tube forward into the right intermediate bronchus the beginning part of the middle-lobe bronchus and the branches of the bottom-lobe bronchus become visible. (Fig. 9.) Also here there is coloration again solely in the area of the carinae;

only with SB 6 is there coloration also of a part of the rest of the ostium region. We cannot say whether and to what extent the precipitate is obtained during the in- or expiratory phase. Heubner is of the opinion -- as mentioned in the introduction -- that the particles probably settle on the carinae on expiration, that is, on the outflow from the narrow into the wide tubes. Fig. 10 shows once more the partition region of the bottom-lobe bronchus and the beginning portion of the middle-lobe bronchus in the vicinity. On accurate observation it is noted that the streak-like marking on the carina of SB 6 is clearly wider than that on the middle-lobe carina; the same holds true for SB 7 and SB 10, even if somewhat less markedly. This is linked with the differing angles of flow-path change of these bronchi, that is, more precipitate is obtained at the point where the branching bronchus goes off in a large angle, and vice versa. Anacker has determined the angle of flow-path change of the lobe- and segmental-bronchi bronchoscopically and came, on the basis of observations, to the same finding as Findeisen, to whose paper Anacker's data are appended (Figs. 3 and 4). In Fig. 11 the right top-lobe bronchus is shown with its typical division into 3 segmental bronchi. On the rear wall where a folding mark is brought out, but also on the medial and slightly on the lateral side-wall, longitudinal streaks are to be seen, and also hints of spirally arranged color precipitates leading to the carinae of the segmental ostia. Also, in the ostium of SB 2 and SB 1 somewhat more dye is noted than in SB 3, which can again be brought into correlation with the differing flow-path change angle.

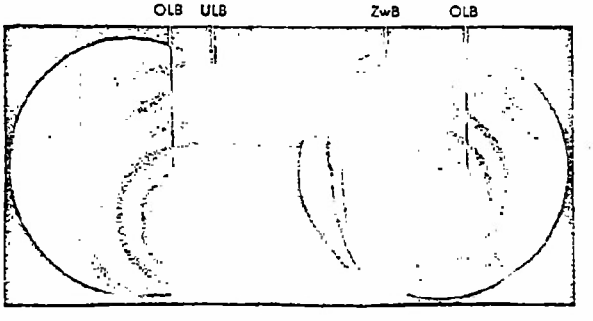

Fig. 8. Left and Right Main Bronchus and Its Partition into Top- and Bottom-Lobe Bronchus (OLB and ULB) resp. Intermediate Bronchus (ZwB) Photographed with the 60° Lens from a Point Slightly Above the Carina Tracheae. In this case, too, dye is to be seen only on the

carina ZwB/OLB (right picture), but the dye continues in the OLB in a ringlike manner. On the original picture one can see on the carina OLB/ULB (left picture) also dye in the form of a streak.

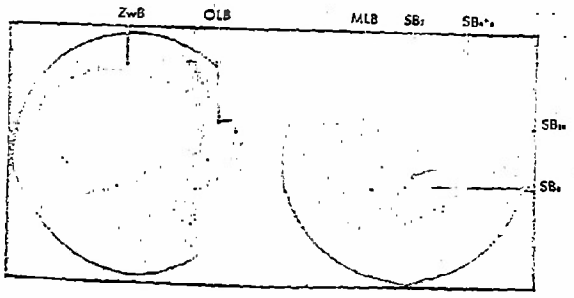

Fig. 9. Right Bronchial System: Intermediate Bronchus with Departure of Top-Lobe in the Left Picture and Middle-Lobe Bronchus (MLB) with Bottom-Lobe Bronchus (ULB) resp. Its Partition into the Segmental Bronchi (SB 6-10) in the Right Picture. Also in this case the dye covers all carinae of the lobe- and segmental-bronchi in streaks and spreads only slightly over to the rest of the ostium region (mouth, common origin of a bronchus).

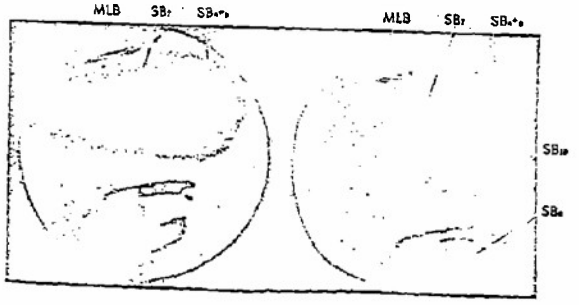

Fig. 10. As in Fig. 8, Right, Closeup of Partition of the Bottom Lobe. It can be seen that the dye streaks from the carina SB 6/UL-Base (at 6 o'clock) is much broader than on the carina MLB/ULB (at 11 o'clock). It is also somewhat broader on the carina SB 7/SB 8 + 9 (at 12 o'clock) than on the carina SB 8 + 9/SB 10 (at 2 o'clock).

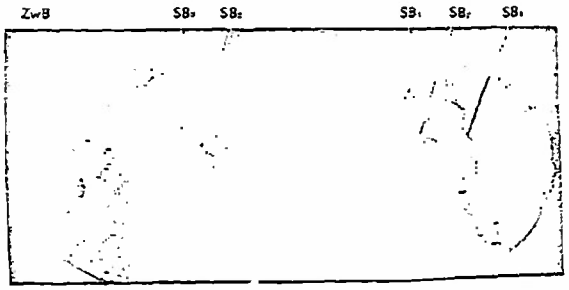

Fig. 11. Right OL Bronchus with Division into the 3 Segmental Bronchi, Photographed with the 60° (left) and 90° (right) Lens. Here the main precipitate is to be seen also on the carinae, and in addition a longitudinally streaked, partly also spiral design may be noted on the wall sections. In addition there is a somewhat larger dye precipitate in the Ostium of SB 2 and SB 1.

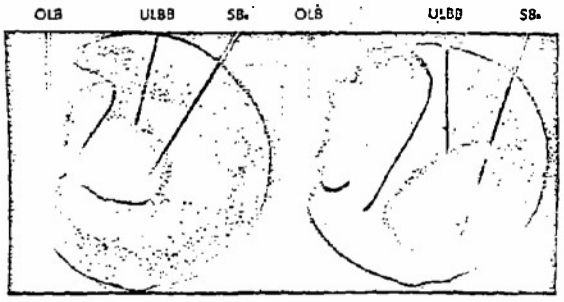

Fig. 12. Lower Left Main Bronchus with Division into OL- and UL Bronchus resp. Here Again into Bronchus of the UL Tip (SB 6) and UL-Base Bronchus; Right Picture a Closeup of the Left. In this case, too, streaky design on the lobe- and segmental-carinae and in addition two longitudinal streaks on the wall of the OL bronchus.

The question arises whether the observed precipitate is a true one or the result of a ciliary stream. In any event we have not been able to observe any dye transport, not even after a one-minute long inspection. Also, even though a few minutes had elapsed between the investigation of the left and right bronchial tree, there were not noted

any essential differences in the inhalation picture either in the carinae or in the wall sections. Probably due to the previous premedication and eventually also through the introduction of the tube a paralysis of the ciliated epithelium had set in, since according to animal-experimental investigations the transport velocity is quite considerable (1-2 cm/min for foreign substances, Antweiler). In our case the precipitate in the partition area of the left upper- or bottom-lobe bronchus (Figs. 12 or 13) was as in the right one: streak-like mark on the carinae of the lobes and segmental ostia, and longitudinal, partly curved lines in the upper-lobe bronchus and its partition region.

It is readily noted (Fig. 13) that very many more color paths are present in the bronchus of the ascending segmental group (SB 1-3) than in the lingula bronchus, which we again bring into correlation with the difference in the flow-path change angle.

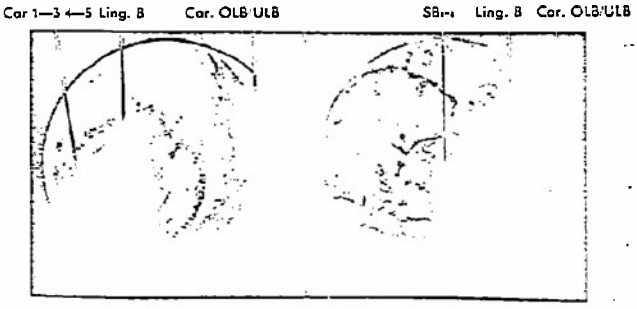

Fig. 13. Division of the Left OL Bronchus into Lingula Bronchus and Bronchus of the Ascending Segmental Group (SB 1-3) with the 60° (left) and 90° (right) Lens. The increased vessel design and the slight angular forward arching indicate slight extramurally determined pathological alterations. In addition to the already described streaky design on the carina there appear in this case more longitudinal, partly arched lines which are found above all in the bronchus of the ascending segmental group.

Influence of cough spasms on the inhalation picture (Fig. 14): We have already emphasized that the subject must not cough either during the inhalation of the dye nor afterwards, particularly during bronchoscopy, since otherwise the characteristic inhalation picture is blurred due to

colored masses of secretion from the periphery. Fig. 14 shows the difference after light (left) and subsequent stronger coughing (right). The main amount of the inhaled dye thus actually lies in the periphery. We thus have an indirect proof that drops of 2 μ in diameter reach almost exclusively the smaller bronchi and the alveoli.

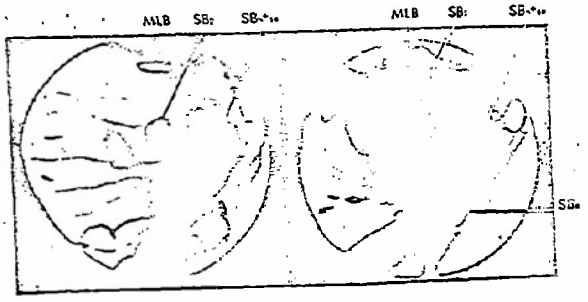

Fig. 14. Intermediate Bronchus with Ostium of the ML Bronchus and Ostia of the UL Bronchus (SB 6-10) After a Slight Cough (left) and a Further, Stronger Cough (right). The black spots and the bands are colored secretion originating from the periphery.

Fig. 15. X-Ray Picture: Darkening of the Right Middle- and Bottom-Field with Slight Displacement of the Heart Due to a Hemorrhagic Pleural Effusion.

Pleural effusion with action on the bronchial system (Figs. 15 and 16): R., J. N., 51 years old, male. X-Ray picture: See Fig. 15.

5 minute inhalation of a 5% Evans-Blue solution. Amount of respired air per minute 10 liters; respiratory rate 12 per minute; dye supported without irritation. Case 3, Figs. 16 and 17.

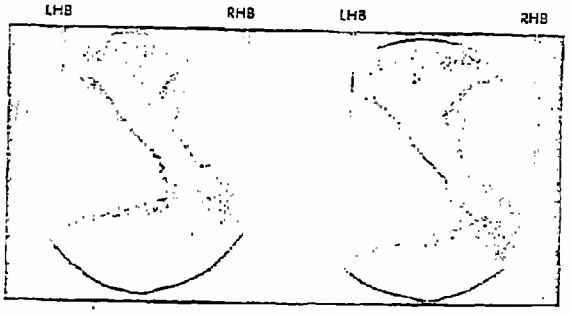

Fig. 16. View From the Lower Trachea at the Bifurcation. The carina is situated atypically on a 11/5 inclined position; left and right main bronchus are visible (LHB and RHB). Dye deposit in the region of the back wall of the trachea exclusively left; front surface of carina strikingly free. By contrast, strong dye concentration on the left side surface of carina.

Corresponding to the compression of almost the entire right lung with rotation and constrction of the right tracheobronchial system, the bifurcation of the trachea occupies a 11/5 individual position; the carina is widened (Fig. 16). It does not contain any dye on the front surface, but on the contrary an abundant amount of same on the left side wall, which displays a dimple-shaped deepening. Apart from this, there is noted a coloration of the back wall of the trachea, however, only in the left section and on the front wall of the left, possibly also the right main bronchus. Fig. 17 shows the conditions in the two main bronchi from close. It can be seen, above all to the left, that the dye is situated in a band-like manner between the cartilage rings, thus in the transverse deepenings, and that it seemingly originates from the site of transition of the medial side wall to the back wall.

The lack of a precipitate on the front surface of the carina, the strong coloration of its left side wall

and the left main bronchus must be related to the special flow conditions resulting from the rotation of the bronchial system and lumen changes in various sections, resp. differing ventilation. Moreover the transverse, band-like dye bands on the front side of the two main bronchi, above all on the left, were probably caused in such a way that the layers of air near the wall, streaming ahead, are blocked by the prominent cartilage edge whereby a vortex formation is brought on and the dye precipitates in the deepenings.

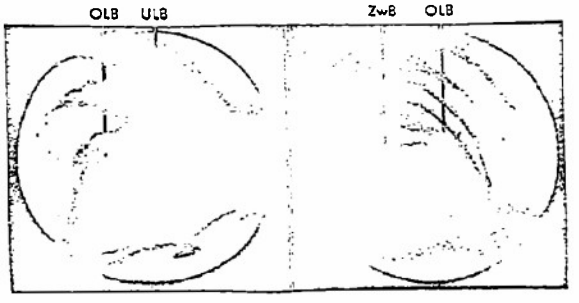

Fig. 17. Left and Right Main Bronchus with Partition in the Depth, Photographed with the 60° Lens. Bands of dye deposit may be seen between the cartilage rings apparently originating from the site of transition of the medial side wall into the back wall. On the left much more than on the right.

Condition of patient: Bilateral collapse operation due to pulmonary tuberculosis; lung-shrinkage- and expansion with distortion, narrowing and expansion of the tracheobronchial system (Figs. 18-23). P. A., 30 years old, female. X-Ray picture: Fig. 18.

Prior to bronchoscopy, 5 minute inhalation of a 5% Evans-Blue solution through a mouth piece. Amount of air breathed per minute: 5 liters; respiratory frequency 10 per minute; supported dye without irritation.

Already at the edge of the tongue, soft gums and on the back wall of the throat abundant dye. The vocal cords are free, with the exception of a mild coloration on the left (Fig. 19). On the other hand, one notes on the dorsal section of the laryngeal entrance a wide, transverse band. From this there extend to the back wall of the upper trachea two parallel streaks, and on the left side wall on top a further dye streak in the lower trachea, and from there in

the two main bronchi (Fig. 20). It is clearly seen that the secretion paths located on the back wall extend exclusively to the right main bronchus and that on the left side wall and to the left main bronchus, while the carina lying in between is colorless. When one follows the dye covering further in the right intermediate, middle- and bottom-lobe bronchi (Fig. 21), one notes in the transverse-oval, stenosed ostium of SB 6 some blue coloration; on the carina and wall of the middle-lobe bronchus a thin streaky layer, while on the other hand in the wall sections of the bottom-lobe bronchus wide, partly circular, partly longitudinal secretion paths which extend into the segmental bronchi. As expected almost all the dye in the bottom-lobe bronchus lies on the left side (Fig. 22), since the top lobes are largely excluded from ventilation due to compression and shrinking. The thick secretion path entering the main bronchus (Fig. 20) is divided into two side paths (Fig. 22) which extend toward the depth chiefly at the transition points of back-wall/side-wall, and enter exclusively into the bottom-lobe bronchus. On closer inspection of the two upper-lobe bronchi (Fig. 23) one notes solely on the left, on the carina upper lobe/bottom lobe bronchus some streaky deposit and on the right in the almost fully stenosed upper-lobe ostium a smaller dye spot.

Fig. 18. Thoracic X-ray Picture with Contrast Representation of the Tracheobronchial Tree. Darkening of the left tip-upper field due to Perlon filling, and of the

right tip-upper field due to extrapleural oleothorax. In the left bottom lobes pronounced cylindrical bronchial ectases; the rest of the tracheobronchial tree is deformed, partly stenosed and considerably displaced.

Case 4, Figs. 19-23.

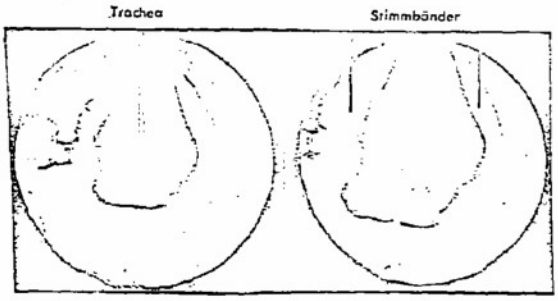

Fig. 19. View of the Vocal Chords and the Upper Trachea by Means of the "Straight-Ahead" Lens. Slight dye deposit solely on the left vocal chord. On the doesal section of the laryngeal entrance wide, transversely deposited band from which colored paths of secretion draw on the back wall of the trachea toward the depth. [Stimmbander = vocal chords.]

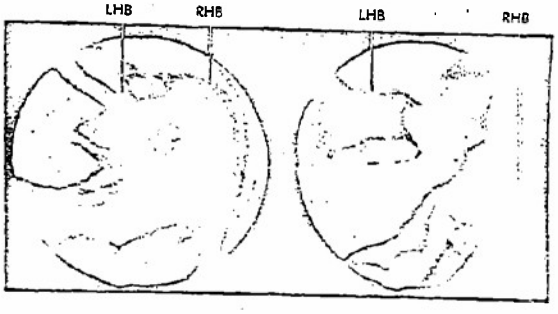

Fig. 20. In the Left Picture the Middle Trachea is Shown with the Two Main Bronchi; in the Right Picture the Lower Trachea with the Two Main Bronchi. The intensity of the dye streaks corresponds to the extent of ventilation. Carina free of deposit.

This inhalation picture is characterized by the particular anatomical condition of the tracheobronchial tree and the lungs as well as the hypersecretion which originates

from the two bottom-lobe bronchi. It is obvious to assume that the dye particles settle particularly abundantly in the mucus paths which are present, and that they undergo an intimate mixing. On the basis of animal experiments Antweiler and Hilding have described such mucus paths but stated nevertheless that they always leave the carinae free. In our own case this is not so, only when the carinae are deformed. In this case, and of course quite generally one must ask whether it is not the special flow conditions that are the cause of the atypical dye deposits. We know from Rohrer that in the normal tracheobronchial tree streamlined flow prevails. However, already Caemsler has called the attention to the fact that the respiratory-air velocity of 3600 ml air/sec, established by Rohrer as critical value, is much too high, and has set the figure at 485 ml/sec. More recently Deldzer et al have found even lower values, and stated in addition that already in quiet, normal respiration the flow in the upper respiratory passages has a turbulent character. In our normal cases (Fig. 7-13) we found, however, no indication of this, yet we must count, in a pathologically altered respiratory passage, thus in the presence of fold-, edge- and ridge-formations, in the case of strong secretion deposition and in deformation, constriction or widening of the bronchial tree, with the occurrence of vortex formation (Figs. 11-12, 19-23). The extent of the air flow in the bronchi is naturally dependent on the respiratory force of the lung (inspiratory suction, expiratory expulsion). Accordingly in diseases of the breathing musculature, pleura, the lung parenchyma and bronchi the aeration is more or less disturbed (Hypo- and hyperventilation), and so is, as a result, the possibility of aerosol transport.

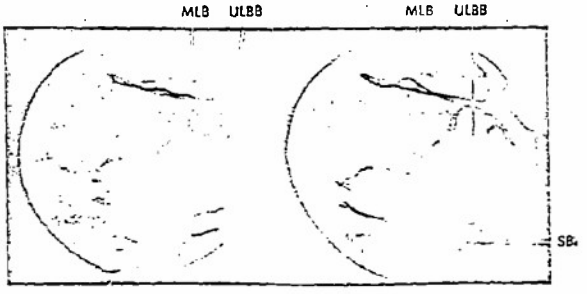

Fig. 21. View from the Intermediate Bronchus into the ML- and UL-Bronchus Right. In the stenosed SB 6 there is a little diffused distributed dye; in the ML bronchus

on the carina and the wall section which is visible, a thin, streaky deposit; in the UL-base bronchus, on the other hand, partly longitudinal thick secretion paths which may be followed into the ostia.

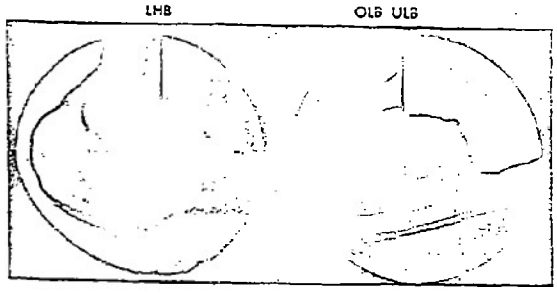

Fig. 22. View into the Proximal Left Main Bronchus (left picture) and the Distal Main Bronchus with Partition into OL/UL Bronchus (right picture). The wide secretion paths draw exclusively into the UL bronchus.

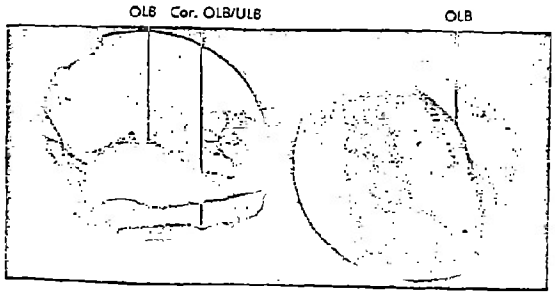

Fig. 23. Markedly Constricted Left OL Bronchus (left picture) and Highly Stenosed Right OL Bronchus (right picture), each Photographed with the 90° Lens. On the left, only on the carina OLB/ULB is there a dye streak; and on the right only a dark spot is noted in the stenosis. [Cor. misprint for Car. = carina.]

Reactivated cavernous tuberculosis in left top lobe (Figs. 24-26): S. C., 31 years old, female; X-ray: see Fig. 24.

5 minute inhalation of a 5% Evans Blue solution; volume of air breathed per minute: 9 liters; respiratory rate 10/min; supports dye without irritation.

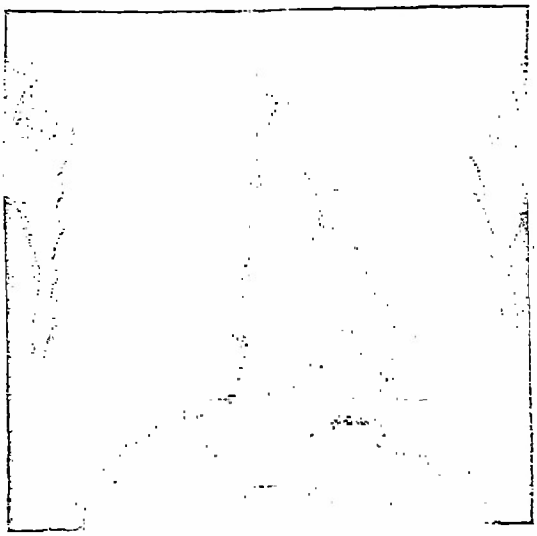

Fig. 24. X-Ray Picture: In the Left Lateral Top Field Tangerine-Size Caverns with Infiltration of Surroundings, Streaky Design Toward Hilus Pulled up High. More Recent Focal Points of Infection in Left Bottom Field, and Older Foci in Right Middle Field.

On observation of the main bronchi one sees a narrow dye path in the right main bronchus, extending into the top-lobe bronchus. The wall of the left main bronchus is free from dye. In the ostium of the left top-lobe bronchus, on the other hand, there is seen, in addition to dye blotches, a purulent mass (Fig. 25) that is not stained. Only the mucus located on its base is colored somewhat bluish. On the original photograph there is to be noted, besides, in the top-lobe bronchus and its extension region an abundant dye deposit. On closer inspection of the top-lobe bronchus by means of a 60° (left picture) and a 90° (right picture) lens (Fig. 26) on again notes, in addition to small purulent clumps, the large pus-containing mass at the base. It is clearly seen that the dye has not mixed with the pus. The more mucous secretion in the vicinity lying above all in the region of the ostium is, on the other hand, clearly stained. In addition in the depth of the top-lobe

bronchus one observes the division of the latter into lingula bronchus and bronchus of the ascending segmental group (SB 1-3). Here, the dye is located mainly in the lingula bronchus, since as a result of the cavernous process in the region of S 2-3 the pertinent bronchial system is poorly aerated. This example points out clearly the possibilities and limitations of aerocol therapy in different bronchial processes and parenchymal processes accompanied by shrinkage.

Case 4, Figs. 25 and 26.

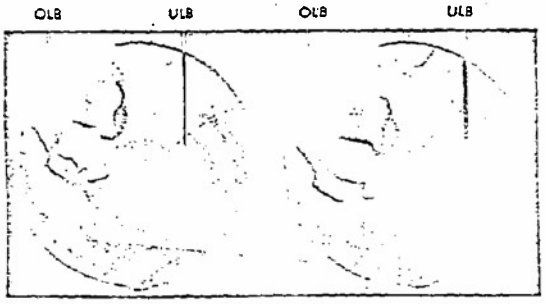

Fig. 25. View from the Lower Main Bronchus into the Left Top and Bottom-Lobe Bronchus. In addition to dye deposit in the ostium of the OLB, there is seen a large purulent mass on the floor. The bottom-lobe bronchus with its segmental ostia is abundantly colored.

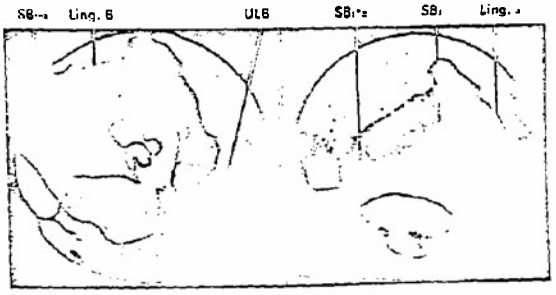

Fig. 26. Left Top-Lobe Bronchus with its Branching into Bronchi of the Ascending Segmental Group (SB 1-3) and Lingula Bronchus (Ling. B), Photographed with the 60° (left) and 90° (right) lens. In addition to the large purulent mass at the base, there are noted smaller pus-containing masses that are not stained; only at the transition to the mucosa is there a slight staining. Abundant staining of the mucous secretion distributed in spots mainly in the ostium. In the ostium of the Ling. B. more dye design than in SB 1-3.

Our investigations performed with colored liquid particles of 2 μ diameter have essentially confirmed the theoretical assumptions of Findeison, Landahl, Anacker, and the glass-model and animal experiments of Heubner. Thus, apart from pathological conditions, particles of this diameter reach mainly the deep sections of the bronchial tree and the alveoli. Trousseau was right when he said 100 years ago: "The droplets penetrate into the lungs; they penetrate much too deeply."

In the short period of this lecture we could not offer further examples of pathological bronchial- and lung-changes which alter the inhalation picture in a typical manner. Also our interpretations must be taken with a certain caution, since it is necessary to carry out supplementary investigations aiming at the clarification of certain questions in order to confirm what has been stated above. It is also necessary to repeat these same experiments with a larger and smaller particle spectrum in order to prove directly the influence of the particle size on the deposition picture in the inspectable tracheobronchial tree.

The evaluation of our experiments is possible from several points of view:

1) One may study indirectly, that is, on the basis of the mucus-membrane picture at the end of inhalation, the aerodynamic flow conditions in the tracheobronchial tree. It seems that already at normal breathing in the pathologically changed respiratory tract there occurs, in addition to streamlined flow, also a turbulent one which modifies the usual dye-precipitation picture.

2) It is possible to make a rough statement regarding the regional lung function in the corresponding parenchyma units from the intensity of the dye precipitate on the lobe- and segmental-ostia. In a limiting sense, however, it must be said that there does not exist any parallel between magnitude of ventillation and dye precipitate, since the latter is influenced by additional factors such as the angle of change of the flow path. For the sake of completion it must be added that certain authors have recently endeavored to examine the problem of regional lung functions by means of radioactively labeled droplets or gases (Knipping et al; West).

3) Our investigations give a direct insight into the possibility and limits of the aerosol therapy in bronchopulmonary diseases. Through employment of different droplet

spectra it will be possible to make a contribution to the much-discussed optimal particle size for different bronchial sections.

4) Bronchoscopic investigation opens up the possibility of directly examining, by means of dye aerosols or liquid droplets brought directly on the mucosa, the activity of the human ciliated epithelium. The studies carried out so far are all based on animal experiments.

5) The method of dye inhalation offers the possibility of treating, by analogy, questions regarding the conditions of development of bronchial cancer through inhalation of carcinogenic substances. Many authors, among them Anacker, consider the carinae of the segmental bronchi to be the sites of preference.

Since bronchoscopy has become a harmless routine method and the possibility exists for the recording of the observed findings by means of photography, the road is free toward the undertaking of such systematically planned investigations.

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Discussion

Olberg, Wiesbaden:

The anticipated contribution regarding the distribution of inhaled chemotherapeutic agents in thoracic tissues must, unfortunately, be limited to the scope of a contribution to the discussion. The tissue analyses which we are carrying out at the present time could not be completed before the start of the Congress, so that I cannot give you any rounded picture with statistically secured results. Leaning on the presentation just heard, I would not like to omit, however, communicating to you at least the status of our problem.

After we [1] have investigated the conditions of secretion of free isonicotinic acid hydrazide (INH) and metabolites in patients of a nursing home for lung tuberculosis after inhalation and oral administration, and noted a considerable retardation in the excretion of free INH after its administration in the aerosol form (Cedin® with 12.5% free INH), we are now determining the various tissue pictures.

We have available the dissection material of a thoracic surgical clinic. The specially prepared tissues are tested chemically-analytically according to the Nielsch method [2, 3]. We are investigating peripheral lung tissues, bronchus tissues, lymph nodes, cavity walls, etc. On the basis of facts known so far, we had expected to find a different INH concentration in the individual tissues, and this indeed turned out to be the case. Of importance is, for example, the observation that after a two-day interruption of the chemotherapy, when there are practically no detectable blood levels, it was possible to demonstrate -- among other places also in the

cavity wall -- the existence of a surprisirgly high INH level (free INH). We hope that we can issue a further report on the final results.

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Rudiger, Bad Lippspringe:

In complementing the excellent lecture of Prof Messerklinger and the beautiful pictorial demonstration of Dr Schiessle, I would like to show a few mucus-membrane photographs of the nose which we have made during our investigations regarding the etiogenesis of rhinopathia vasomotoria. -- The nose is the point of entry to the respiratory tract. It is here that air impurities are first precipitated, this precipitate releasing, depending on the reaction state [of the organism], symptoms and mucosa alterations which not infrequently exhibit the clinical picture of vasomotor rhinopathy. It would, however, be entirely wrong to explain these partly purely reflexively determined phenomena in every case as an allergic reaction. The term vasomotor rhinopathy characterizes a syndrome that can be quite varied in its etiogenetic interpretation. Permit me to illustrate this briefly by means of a few pictures:

Figures 1-3 show mucus membrane reactions released through indifferent aerogenic dust irritation: the expression of a neuro-vegetative reflex hypersensitivity:

1) The lower nasal muscle in a relatively symptom-free state, but typical pale red hypertrophy.

2) Swelling after dust application.

3) After rinsing out the dust particles, the picture of the post-provocatory rhinopathy.

Fig. 1

Fig. 2

Fig. 3

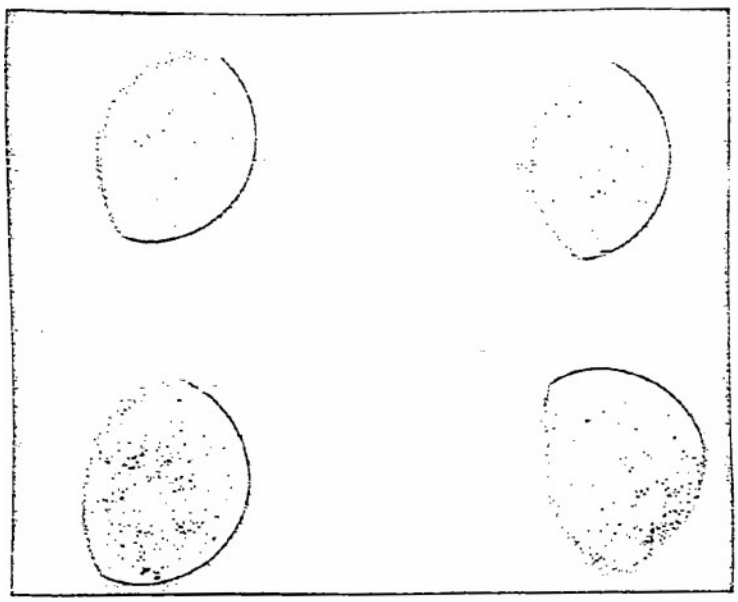

Fig. 4

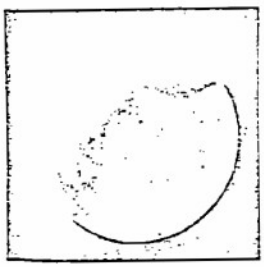

Fig. 5

The next two pictures show in this connection the nasal mucosa of an allergic subject in its reaction to an antigen provocation, in our case 1:10 flour/clay.

4) On the top left, before antigen provocation; top right, 5 minutes later; bottom right, 10 minutes later; bottom left, 15 minutes after antigen provocation.

5) The exit of secretion droplets which in such a case often exhibit the presence of large numbers of eosinophilic cells.

Eichborn, Munich:

As a non-physician and non-biologist it was new to me that in the narrow tubes of the respiratory tract up as far as the lung the flow velocity decreases. From the point of view of flow, this would mean that there must be present there a strong compression. If this were a verified fact, then one would have to draw from it also conclusions regarding the behavior of the aerosol particles, since then the concentration in volume is increased and hence also the sedimentation velocity. This still would have important consequences for the problem of centrifugal ejection again at the rightly emphasized sites where the flow is reversed. But, as I said, the fact is surprising that the flow velocity drops in the small cross sections. This is exactly what would not be expected, since in the case of a constant, stationary flow the velocity would have to be greater in the smaller cross sections.

Dirnagl, Munich:

In regard to the remarks of Mr Eichborn, I would say that the decrease in flow velocity obviously comes from the fact that the number of the individual channels becomes constantly greater, since otherwise the continuity of the flow would not be guaranteed.

I would, however, like to ask Mr Schiessle whether under certain conditions the deposition pictures of the dye could be influenced by resorption or removal of the dye between inhalation and bronchoscopy.

Friedberg, Gottingen:

1) I would like to ask Mr Schiessle whether in his opinion the deposition takes place primarily or secondarily. We, too, have seen the deposition in the carinae, and, in

fact, in experiments with rats which we had treated with graphite powder. When we then prepared the animals after many hours and made their lungs transparent according to the Spaltholz method, we saw that these insoluble particles had been pushed together at the carinae.

2) I would also like to ask whether the employment of Evans Blue is quite harmless. Doubtless Evans Blue is considered a harmless dye. We have, however, performed experiments in Gottingen regarding lung purification and dust elimination and ascertained that it is precisely through this dyestuff, when it reaches the depths of the lungs as an aerosol after the inhalation of dust, that one is in a position to so damage the alveolar epithelia that the dust elimination is considerably restricted.

Schiessle, Freiburg:

The first question by Mr Eichborn was settled by Mr Dirnagl. It indeed so happens that the total cross section increases in the depth as otherwise, as Mr Dirnagl state, a flow in the depth would be quite impossible.

Now, to Mr Dirnagl's question. He asked whether there occurred a resorption already between the end of inhalation and the bronchoscopy. This is by all means possible although it must be assumed that the colloidally suspended dye does not pass through the bronchial wall without any further ado. Since we performed the bronchoscopies for diagnostic reasons, we have collected tissue material for the histological investigation at different places. Mr Konn of the Freiburg Pathological Institute has paid particular attention to the dye content of the tissue. He found some dye only on the upper surface in the mucus insofar as the latter had not been eliminated through the working up of the material, and occasionally also some dye in the epithelium. In the deeper layers, no blue coloration was ever noted. Already in 1953, we had carried out animal experiments with the same dye and noted an intercellular advance. In these experiments, however, the supply was much greater, and besides, the animals inhaled for 1/2 hour. As was reported, in our present investigation the duration of inhalation lasted only 5 minutes. One can thus say that there does not take place any histologically demonstrable resorption worth mentioning.

To Mr Friedberg: I, too, am familiar with the fact that Evans Blue is not entirely harmless. It is, in fact, considered as a vital stain, and is often used for the determination of blood volume, but in recent times voices

have been raised on various sides against its employment. I have first begun my investigation with Trypan Blue, but soon went over to Evans Blue due to the more intensive contrast of the latter. As I mentioned in the lecture, in some patients certain bronchial irritation phenomena had set it in the form of throat clearings and short coughing spells, but we have noted such occurrences also in inhalations with medicaments, particularly when a bronchitis is present. The first question, whether the bronchoscopically ascertained deposition takes place primarily or secondarily, I cannot answer with certainty. On the basis of our observations, we believe that as a result of the paralysis of the ciliated epithelium no dye transport sets in and hence the observed precipitate is a true one. One must consider as a main source of disturbance any coughing, no matter how mild.

Translation No. 858

INVESTIGATIONS ON THE ABSORPTION OF AEROSOLS IN ANIMALS

ON THE INHALATION OF THE FINEST AEROSOLS A COMPARATIVE STUDY ON SMALL RODENTS

by Dr H Oldiges

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Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; pp 52-55

As industrialization increases, the atmosphere is becoming more and more enriched in suspended particles. Although a differential filtration technique allows the purification of, say, industrial waste gases from coarser admixed substances, the finest aerosol fractions nevertheless are carried into our environment in not inconsiderable quantities. To this is added, under certain circumstances, the contamination through radioactive components which are supplied during atomic processes and nuclear explosions. Here it is again the finest portions which, in the long run, endanger wide areas due to their low sedimentation velocity. The physical and chemical actions of the finest aerosols on the biological events in the organism require, however, a thorough clarification. It is thus advisable to devote particular attention to this portion of suspended particles. By means of exhaustive experiments on rats, mice and gold hamsters, it was attempted to obtain a general view of the quantitative absorption of the finest liquid aerosols of the aqueous phase.

Material and Method

The animal material used in these inhalational experiments was of uniform origin and quality. The average weight of the rats amounted to 208 g, that of the gold hamsters 62 g

and of the mice 23 g. The average fresh lung weights of rats, hamsters and mice were 1.5, 0.6, and 0.25, respectively; while the trachea weights were 0.14, 0.07 and 0.03 g, respectively.

All experiments were carried out at a room temperature of 21° and a relative humidity of 63%. For aerosol source we had available the well-known ultrasonic atomizer which produces an aerosol by means of a focussed barium titanate vibrator on the liquid surface. For sonic frequency we chose 2.7 kilocycles. A continuous oxygen current of 2L per minute carries the relatively homogeneous aerosol into a glass bulb, through a piping system. On this bulb was erected a Liebig condenser customarily used in chemistry; this condenser was connected by means of a tube with a container to which smaller plastic flasks were secured, containing the experimental animals. The glass bulb, the Liebig condenser, and the whole tubing system served for the thorough screening of the aerosols. By means of preliminary experiments and continuous aerosol measurements, it could be demonstrated that the droplet spectrum on the level of the experimental animals and in the region of the experimental chamber remained approximately constant during the performance of the experiment. We had an aerosol available whose largest particles had a diameter not above 1 μ. The most frequent particle size was 0.6 μ. The particle density amounted to 10^6 particles per cm^3 and the mist density amounted to 145 γ methylene blue per liter of aerosol. For the atomization we used a 1.25% methylene-blue solution having a pH of 4.3. Each experimental series was several times repeated with 10 animals each. The communicated values are average values from 4 or 5 experimental series.

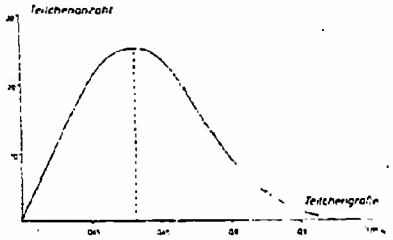

Fig. 1.

1 -- Number of Particles; 2 -- Particle Size.

Experimental results

The investigations of Findeisen, Stieve et al have shown that particles of the order of magnitude used in the

present experiments can enter the lungs and may peretrate as far as the alveoli. After the data of Dirnagl, the main cause of the settling of the finest aerosols in the air passages is the Brownian molecular motion. On the other hand sedimentation plays only a subordinate role. Further there is a close relationship between breathing rate and retention, as demonstrated by Dirnagl and Pichlmeier in humans.

Our formulation of the problem began with our attempt to clarify what quantities of the fine aerosol offered to the organism are actually transported to the lungs and what portion is precipitated already in the trachea. A relatively large portion is already precipitated in the upper respiratory passages since the experimental animals employed breathe through the nose. It should be noted beforehand that the resorption of methylene blue through the respiratory tract can be neglected when the duration of the experiment is two hours, and the animals are killed and immediately dissected. Methylene blue could not be found in the urine in any of the investigated cases, not even as a leucobase.

The breathing rate of a rat is about 120 per minute. With each cm^3 of inhaled air the animals took up 0.145 γ methylene blue. On concentration measurements by means of the spectrophotometer only small quantities of methylene blue were found in the respiratory tract. In the trachea which was cut off up to the bifurcation 11.2 γ methylene blue had been deposited; in the lungs proper only 10.7 γ.

Gold hamsters have a breathing rate of 160 per minute. In this animal species only 6.7 γ methylene blue could be detected in the trachea and 3.6 γ in the lungs.

Mice breathe at the rate of 210 per minute. After an experiment lasting two hours, 5.4 γ substance was detected in the trachea and 2.4 γ in the lung tissue. Here the comparative values between the individual animals are not quantitatively conclusive, since the breathing rate and the dynamics of breathings play a large role, as do the anatomical particularities.

The experimental series with rats, gold hamsters and mice clearly show that as the breathing rate increases there is a marked decrease in the retention of methylene blue. While in the case of rats the amounts of methylene blue deposited in the lung tissues and in the trachea were about the same, in the case of the gold hamsters the amount in the lungs was only a little over one-half that which deposited in

the trachea. With a further increase of the breathing rate as in the case of mice, the portion deposited in the lungs further decreases and no longer attains one-half the amount detectable in the trachea.

The results presented here allow us to draw the conclusion that nasal breathers with a very high breathing rate are only suitable for studies with very fine aerosols whose particle-size spectrum ranges up to 1 μ under certain conditions. A large part of the substance supplied is either retained in the nasal-throat space or eliminated from the lungs through expiration. Only about one-half to one-third of the precipitated substance is separated in the lung tissue and penetrates as far as the alveoli with certainty. This small proportion can under certain circumstances acquire a very great significance when the case is that of a pharmacological or toxic aerosol.

On the basis of these animal experiments which we have extended also to other animal species, we unfortunately reach the conclusion that comparative studies, with different animal species, regarding the effectiveness of aerosols do not allow any comparisons to be made. Not only is there among the different animal species a qualitative difference in the aerosol absorption, but possibly there exists also a preferential absorption for certain aerosol fractions. This obvious difference is determined not only by the physiological condition of the respiratory passages but also by the difference in the mechanism of respiration.

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Translation No. 859

QUANTITATIVE INVESTIGATIONS ON DUST ELIMINATION IN ANIMAL EXPERIMENT*

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Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961, pp 56-74

In the lungs of coal miners who have died during the last decade various authors [1, 2, 3, 4] have found about 10, 20 to 50 g, in rare cases up to above 100 g dust. If such miners had worked for 30 years as coal miners at an average fine dust concentration of 30 mg/m^3, they must have inhaled -- when one adds the dust load of all worked layers -- about 2.5 kg dust. On the basis of the different elimination measurements known from the literature one can assume that out of this amount up to 1.5 kg has again been exhaled and perhaps 1 kg lodged in the air passages in the lungs. A comparison of postmortally found dust amounts with the calculated dust deposition shows that the purification of the lungs is a function of vital importance.

The qualitative aspects of lung purification, that is, the mechanisms and paths of purification, are quite well known [5-11]. Only since the last 10 years has one been exhaustively occupied with the quantitative aspects -- the purification capacity. This subject was first approached by American authors and in fact mainly with the employment of

* The investigations were carried out with the financial support of the High Authority of the Miners Union and the Bochum Miners' Brotherhood.

radioactive dusts (for literature references see Friedberg [12], as well as Klosterkotter and Bunemann [16]). Since a few years some European institutes, too, are working in this area [12-21]. It was Friedberg in Gottingen who has first begun with the exhaustive investigations; a little later we ourselves have followed. We are now going to review results obtained with some 3,000 experimental animals (rats).

When one wishes to make quantitative statements regarding the elimination of inhaled dusts, one must unfortunately point to animal experiments. Insofar as one is interested only in the primary bronchial purification, one can naturally experiment also on humans, for example by means of contrast bronchography. Of much greater interest for pneumoconiosis research, industrial toxicology and safety engineering in the atomic industry is, however, the investigation of the capacity and the temporal course of the purification of the alveolar region. By alveolar region we mean, for the sake of simplicity, the pulmonary sections free of ciliated epithelia, thus, the bronchioli alveolares, ductuli and sacculi alveolares.

However, in such studies one must first of all know how much dust has initially deposited in the lungs after inhalation, and later, how much dust is retained after different experimental periods. Hence, one must kill the experimental animals in groups and analyze the lungs and regional lymph nodes by means of sensitive microbiological methods. The reason why the extrapulmonary regional lymph nodes are of particular interest is that their dust content indicates how much of the deposited dust is removed by the lymphatic system and at the same time indicates whether dust has reached the lungs from the alveolar region in larger amounts.

Before we report on newer investigations performed at our Institute, we will briefly discuss the methods of analysis and the ways in which the dust was applied.

One should naturally employ in inhalation experiments a method of dust application which conforms to current practical conditions as much as possible. Simply put, one can distinguish between three basic processes which lead to the production of dust aerosols:

1) The whirling up of dust particles;
2) The production of fresh dust in various mechanical processes such as grinding, drilling, carving, etc.;

3) The formation of smokes in melting and combustion processes and in electrochemical processes.

We operate with a whirling-up process. The dust is whirled up by compressed air and passed into the inhalation boxes through a tubing system provided with an intermediate separator. Concentration and duration can be varied at random, according to the problem investigated.

The indication of the method of dust application seems to me very important because, for instance, Mr Friedberg [14], using the Polley dust generator in which the dust is produced by means of a grinding process, obtained different results from ours, while our own results agree with those of our French colleagues in Verneuil who also use a whirling-up process.

So far we have experimented with the following dusts: quartz, quartz glass, amorphous SiO_2 dusts of the Aerosil type and silica-gel type, trass, clay slate, gamma-Al_2O_3, TiO_2 and soot. We used the following methods of analysis:

I. Determination of Silicic Acid

according to the method of Carlson and Banks, as modified by Stegemann and Fitzek [33].

The very carefully bled-out lungs are dried in the vacuum drier and then individually decomposed in platinum crucibles with soda-potash. After decomposition, the melt is dissolved in the drier at 80°C with twice-distilled water, and, after cooling, adjusted to pH 6-7 with 1N NCl. Then x ml of the analytical solution, corresponding to an optimal SiO_2 content of 80-160 gamma are pipetted into a 100 ml flask and filled up to 70 ml with twice-distilled water. For the formation of complex the pH is adjusted to 1.1 with 3N H_2SO_4 and treated with 5 ml 8% ammonium molybdate solution. After 15 minutes, depending on the phosphate content, 2.5-5 ml 40% tartaric acid is added through a pipette. The blue color complex is developed by means of an eiconogen buffer mixture at a pH of 1.3. After an additional 20 minutes, the extinction is measured in the photometer (ELKO III) (Filter S 72).

The addition of HF for depolymerization of the silicic acid is unnecessary when the analyses are carried out immediately after the decomposition.

For the sake of comparison we have carried out, simultaneously, a large number of analyses according to the method

of H Baumann [34]. (The methodology is to be looked up in the cited article.) We have obtained according to this process analytical results in close agreement with those of our standard procedure:

Reagents:
1. 2N HCl
2. 3N H_2SO_4
3. Ammonium Molybdate Solution, 8%
4. Tartaric Acid Solution, 40%
5. Eiconogen-Buffer Mixture: 12 g Sodium Pyrosulfite
   2.4 g Sodium Sulfite · H_2O
   0.2 g eiconogen
   85 ml Twice-Distilled Water

II. Determination of Titanium

In the case of titanium quantities of above 150 gamma per lung we work according to the usual H_2O_2-procedure. In the case of smaller Ti content we use a method which we have adapted from LeBouffant [37]:

The lungs resp. the glands are decomposed in a wet process with H_2SO_4 or HNO_3. After the decomposition, the solution is adjusted to a pH of 3-3.2 by means of NaOH and transferred into 50- or 100-ml flasks. Then 20 ml buffer solution, 10 ml ascorbic-acid solution and 2 ml chromotropic acid solution are added. After filling up, the solution is examined photometrically after 15 minutes, at 470 mm μ.

Reagents:
1. Ascorbic acid: 2% aqueous solution
2. Chromotropic acid: 6% aqueous solution. The solution contains, in addition, 4-5% sodium sulfite
3. Buffer solution: 236 g monchloroacetic acid in 300 ml water; 50 g sodium hydroxide in 300 ml water. The two solutions are mixed and made up to one liter. Solutions 1 and 2 above must be freshly made up daily.

III. Determination of Aluminum

A) Determination with Eriochrome Cyanine According to F Richter [35]:

The lungs are decomposed with 500 mg soda-potash. After transfer to 250 ml flasks, the pH is adjusted to 2.2

with 2 ml HCl (1:1). The solution is treated with 10 ml aqueous SO_2 which is then again cooked up (10 minutes). After cooling, it is filled up. Twenty-five or fifty ml of this solution is transferred to 250 ml measuring flasks, treated with one drop thioglycolic acid and one drop phenolphthalein, and then treated with 1N NaOH to a weakly pink color. After addition of 4 ml 0.1 N H_2SO_4 and 20 ml dye solution (0.1% aqueous eriochrome cyanine solution*), the samples are allowed to stand for 20 minutes. Then 50 ml acetate buffer is added and the flask is filled up.

After four hours, photometry: Hg filter 546; layer thickness d = 1.

Solutions: 1. 1:1 HCl
2. 5% SO_2 in H_2O
3. 80% Thioglycolic Acid solution
4. 1N NaOH
5. Acetate Buffer, pH 3.8; 32.7 g sodium acetate, 105 ml 96% acetic acid made up to 5 liters with distilled water
6. Dye solution: 0.1% aqueous eriochrome cyanine solution.

B) Determination of Aluminum with 8-Hydroxyquinoline According to C R Gentry and L G Sherrington [36]:

The method is suitable for amounts 5-10 gamma aluminum. The decomposed lungs resp. glands are transferred to 50 ml flasks and after addition of 2 g KCN heated for 5 minutes to 50°C. Then 10 ml sodium sulfite solution is added. After cooling the solutions are made up to 50 ml and transferred to a 500 ml separatory funnel. After the additon of ammonium nitrate to a pH of 9-11, it is extracted with 10 ml oxine-chloroform solution. Before the measurement of the extinction at a wavelength of 395 mm μ the chloroform phase is dried for five minutes over sodium sulfate.

Reagents: 1. 1% solution of 8-hydroxyquinoline in chloroform
2. 10% sodium sulfite solution.

For experimental animals we used white rats of the same breed, weight and sex, mainly female. We would like to state beforehand that in our experiments male or female rats showed

* The eriochrome cyanine solution should be four weeks old at the time of application. Under these conditions its color intensity remains constant for longer periods of time.

no difference in regard to the rate of elimination, nor were there noted any significant breed differences between our own breed and Sprague-Dawley rats. Larger rats, however, absorb, under the same conditions, more dust than smaller ones, and they eliminate dust somewhat better. Thus, the weight does play a role in regard to deposition, as was expected.

Results

We would like to divide the results according to the investigation of the quick phase and the slow phase of dust elimination. The quick phase corresponds to the primary purification of the bronchi and bronchioli through the action of the ciliated epithelium; the slow phase is characterized by the purification of the alveolar region, protracted over a longer period of time by means of the bronchial path and through the pulmonary lymph vessels.

In regard to the quick phase of purification, we have performed the following experiments:

Immediately after killing the rats we prepared out the lungs including the trachea and suspended the surviving lung preparation on the glottis in a humid chamber. Thereupon, we injected in the periphery of the right or left inferior lobe 0.05-0.1 ml India ink and measured the time elapsed between the injection and the appearance of the first trace of India ink in the region of the bifurcation tracheae. The time of transport lasted in the various experiments 10 to 18 minutes, that is, the first portion of India ink appears at the bifurcation after this period of time. The transport from the bifurcation to the glottis lasted 2 1/2 to 8 minutes. In the case of a tracheal length of about 2 1/2 cm in the suspended state, this means a transport velocity of 0.3 to 1 cm/min.

The action of the ciliated epithelia thus causes in rats a quite rapid dust transport (cf Antweiler [9]).

From these results it is to be concluded that during a longer experiment a large part of the deposited substance is eliminated already during the inhalation. Unfortunately, this amount cannot be determined in the living animal, nor even approximately calculated. It is with this limitation that one should evaluate the following results as well: We allowed 30 rats to inhale for four hours a Dorentruper quartz powder No 12. Immediately after they were taken out of the boxes and also 4 and 24 hours later 10 animals each were killed and the lungs analyzed for SiO_2. In the lungs of the immediately killed animals we found on an average 150

gamma SiO_2; after 4 hours about 30% of this amount had been eliminated, and after 24 hours about 42%. The same experiment with an amorphous SiO_2 dust had the same course (Fig. 1). Collet [22] found in animal experiments with TiO_2 an elimination of 45% in 6 days, and Fish [23] observed after inhalation of U_2O_3 an elimination of 50% in 6 days. Bair [24] obtained in experiments with plutonium in 2 weeks an elimination of 52% through the intestine. In the case of insoluble dusts, however, the enteral elimination may be considered a good measure of the bronchiogenic purification, since the dust is generally absorbed after reaching the glottis.

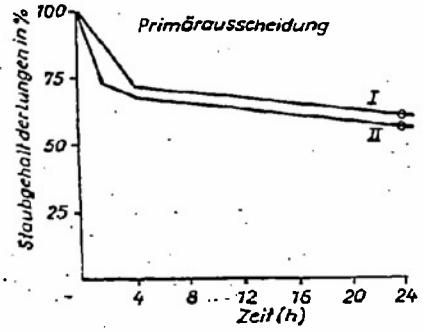

Fig. 1. Separation of Quartz and Amorphous SiO_2 within 24 Hours Following Single Inhalation. Primary Separation.

I = Quartz 100% = 0.150 mg
II = SiO_2, amorphous 100% = 0.194 mg

1 -- Dust Content of Lung in %; 2 -- Primary Separation; 3 -- Time (hrs).

Our primary elimination curves show after 2 resp 4 hours a sharp break. Thus, during this time the quick phase of bronchial purification is essentially completed. Naturally, one must assume that the values may vary for the primary bronchial purification in function of the particle size spectrum and the duration of inhalation. The larger the size of particles or aggregates deposited the greater is the percentage of the quickly eliminated dust.

The quick purification phase must be taken into consideration in the evaluation of the inhalation of toxic dusts. In general, the inhalation is particularly dangerous, since there occurs a rapid resorption in the lungs. While earlier the oral absorption and enteral resorption had been considered particularly important, for instance in lead poisoning, today the greater significance is assigned to the inhalation of

lead dust and lead smoke. The same holds true for arsenic with which we have been occupied over a longer period of time. One could enumerate a whole series of additional substances which occur as individual poisons in the form of dust or smoke.

The experimental results regarding the quick phase of purification indicate that at least 1/2 of the solid substances deposited in the respiratory passages reach the gastrointestinal tract within a few hours. In this region the solution conditions prevailing are different from those in the lungs -- mostly better. The arsenate luminous substance with which we have experimented was practically insoluble at a tissue-pH of 7.3, but fully soluble in the gastric juice. We had to calculate the danger through inhaled arsenate powder essentially as if it would be absorbed. Of the retained half of the substance an additional, considerable amount reaches the gastrointestinal tract after a longer period of time unless the velocity of solution is great enough for a resorption to take place in the meanwhile in the lungs. The lung can thus act to a certain extent as a poison depot from which the gastrointestinal tract is slowly supplied. This is so since a quick pulmonary resorption only occurs with liquids and highly soluble solids, particularly when the molecular weight of the solutes is less than 20,000. In these cases, according to Ruszniak, Foldi and Szabo [25] there occurs an immediate resorption; products having a higher molecular weight are resorbed lymphogenically.

Now let us turn to our own investigation of the slow phase of dust elimination. We have seen that the quick phase is completed after 24 hours. Accordingly, we always set as the initial value in the observation of the slow phase the retention value analyzed in 10 rats 24 hours after the termination of inhalation = 100%. Additional groups of animals are killed after one and more months. So far we have obtained elimination curves up to one year. The values given in the following table are averages for 5, in most cases, however, for 10 analyzed lungs. They give the dust content in percent of the initial values.

I would like, first, to briefly summarize earlier experimental results on which we have already reported in Oxford and Paris [26, 27].

In a great number of experimental series we repeatedly observed that the percentual elimination decreases as the 24-hour retention value increases (Fig. 2). The absolute elimination, however, increases proportionately and becomes quite

high -- in some experiments the increase is 50-fold. The lung purification thus possesses a wide adaptive latitude.

TABLE 1

Elimination of Quartz after a Brief Period of Inhalation. Increase of the Absolute Quartz Elimination with Increasing 24-Hour Retention Value

Quarzelimination in absoluten Zahlen

Retention (γ) Ausgangswert	Davon ausgeschieden nach (γ) 3 Mon.	5 Mon.	8 Mon.	12 Mon.
177	—	—	127 (72%)	
180	99 (55%)	115 (64%)	—	—
500	256 (53%)	300 (60%)	—	—
562	—	—	—	387 (69%)
915	329 (36%)	366 (40%)	—	—
1870	—	—	—	1197 (64%)
5360	1768 (33%)	—	—	—
9960	1496 (15%)	3785 (38%)	5079 (51%)	—

1 -- Quartz Elimination in Absolute Figures; 2 -- Retention (gamma) Initial Value; 3 -- Of this Amount, Eliminated (in gammas) after; 4 -- Months.

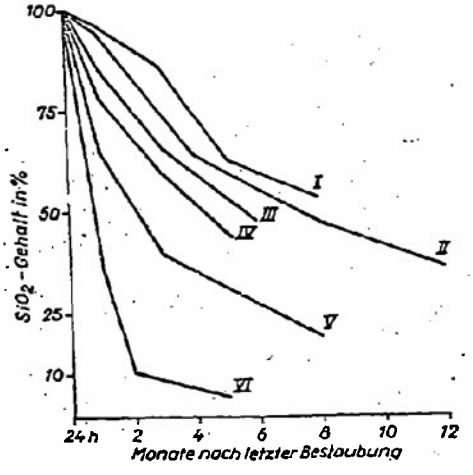

Fig. 2. Elimination of Quartz After Short-Period Inhalation at 24-Hour Retention Values of 9.96 to 0.19 mg/lung. Decrease of the percentual elimination with increasing initial value.

I 100% = 9.96 mg; 8 X 6 hrs. consisting of quartz
II 100% = 1.94 mg; 4 X 6 hrs. consisting of quartz
III 100% = 0.64 mg; 2 X 6 hrs. consisting of quartz
IV 100% = 0.33 mg; 1 X 6 hrs. consisting of quartz
V 100% = 0.19 mg; 1 X 2 hrs. consisting of quartz
VI 100% = 0.84 mg; 8 X 6 hrs. consisting of Aerosil
(Months after last application of dust)

1 -- SiO_2 Content in %; 2 -- Months After Last Application of Dust.

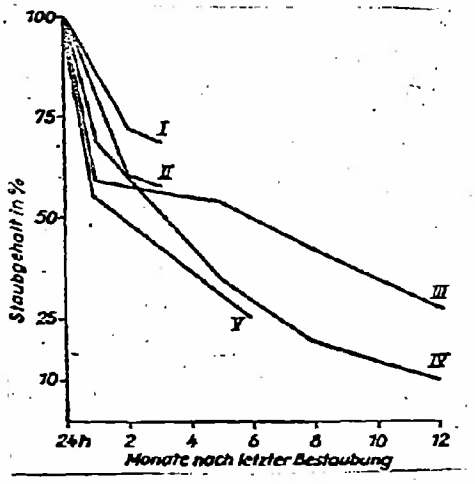

Fig. 3. Elimination of gamma-Al_2O_3 (Particle Size 5-50 mμ) and TiO_2 (Particle Size 0.05-0.2 μ) After Short-Term Inhalation.

I γ-Al_2O_3 100% = 1.590 mg
II TiO_2 100% = 5.670 mg
III Al_2O_3 100% = 0.732 mg
IV TiO_2 100% = 0.435 mg
V TiO_2 100% = 0.920 mg

1 -- Dust Content in %; 2 -- Months After Last Application of Dust.

The rate of elimination is, during the first month, still relatively high in most cases; however, it then becomes increasingly smaller, the curves become even more horizontal. This holds true for all difficultly soluble dusts such as quartz, Al_2O_3 and TiO_2 (Fig. 3). The dust elimination after a single or brief period of inhalation is a relatively slow and incomplete process. For quartz we could demonstrate that after a few months, up to one year, 20 to 50% of the

TABLE 2

Quartz Elimination and SiO_2 Content of the Regional Lymph Nodes After Short-Term Inhalation

Staub	Retention γ/n. 24 Std.		Elimination und Lymphdrüsenwerte			
Quarz	177	a)	1	3		
		b)	39	97		
		c)	7	6		
Quarz	415	a)	1	3	4	5
		b)	151	209	236	265
		c)	13	20	43	43
Quarz	562	a)	1	3	7	12
		b)	196	308	336	389
		c)	16—92	70—147	31—170	80—245
Quarz	636	a)	1	3	6	
		b)	95	139	436	
		c)	43	104	121	
Quarz	767	a)	3	7		
		b)	254	330		
		c)	45—214	30—170		
Quarz	915	a)	1	3	5	
		b)	301	327	386	
		c)	56	104	213	
Quarz	1870	a)	12			
		b)	1193			
		c)	481			
Quarz	1935	a)	1	4	8	12
		b)	—	865	1017	1216
		c)	116	234	408	530
Quarz	5470	a)	2	3	5	
		b)			1585	
		c)	160—492	160—725	334—462	

a) Time in Months
b) Eliminated from the Lungs (average values in gammas)
c) Found in the Mediastinal Lymph Nodes (in gammas)

1 -- Dust; 2 -- Retention, gamma per node, 24 hours; 3 -- Elimination and Lymph-Gland Values; 4 -- Quartz.

amount which had disappeared from the lungs was deposited in the mediastinal lymph nodes, that is, eliminated lymphogenically (Table 2). By contrast, in the case of TiO_2 and Al_2O_3 only traces or at the very most 10% of the eliminated amount is found in the extrapulmonary regional lymph nodes. This quartz exhibits a pronounced lymphotropism. I recall, in this connection, the findings of Sano and Osanai [31]. These authors differentiate between an alveolar and a lymphatic type of pneumoconiosis. Silicosis belongs to the lymphatic type, while in the case of aluminum dust in the lungs the lymph glands, as is well known, are hardly involved.

We have already pointed out that the elimination curves of the more readily soluble amorphous SiO_2 dusts of the aerosil type basically differ from those of the silica gels. Such dusts are eliminated to a great extent within 1-2 months, but residues are detectable in the lungs for a long time and in the lymph nodes only a relatively small amount is found. A comparison with the elimination curves of Al_2O_3 which has the same particle size as aerosil, indicates that in this quick elimination the particle size cannot be of any great significance (Fig. 4). There nevertheless exists a marked correlation with the solution velocity. Here it is again noted that the more insoluble SiO_2 dusts are eliminated more slowly than the more soluble ones (Fig. 5). (KS 300 [silicic acid?] and KS 300/600°).

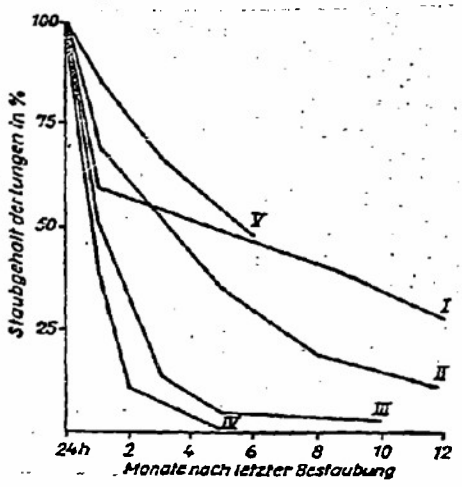

Fig. 4. Elimination Curves of gamma-Al_2O_3 and TiO_2 in Comparison with Amorphous SiO_2 Preparations (Aerosil and Precipitated SiO_2). The primary particle size of Al_2O_3 and SiO_2 Preparations is about the same.

I	γ-Al_2O_3	100% = 0.732 mg
II	TiO_2	100% = 0.920 mg
III	SiO_2	100% = 0.584 mg
IV	SiO_2	100% = 0.841 mg
V	Quartz	100% = 0.640 mg

1 -- Dust Content of Lungs in %; 2 -- Months After Last Inhalation of Dust.

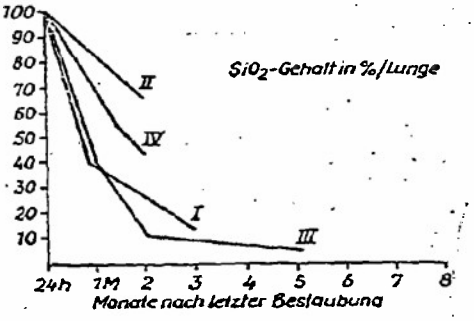

Fig. 5. Elimination Curves of Amorphous SiO_2 Preparations with Differential Solution Velocity.

I	KS 300 normal	100% = 1.17 mg 81 γ/ml/24 hrs.
II	KS 300 600°C fused	100% = 1.15 mg 33 γ/ml/24 hrs.
III	Aerosil	100% = 0.84 mg 27 γ/ml/24 hrs.
IV	Aerosil, coarse	100% = 1.38 mg in Aqua dest.

SiO_2 content of lungs in %

1 -- SiO_2 Content of Lungs in %; 2 -- Months After Last Inhalation of Dust.

Of interest also is the fact that amorphous quartz glass is better eliminated than quartz. We had suspected this already earlier on the basis of histological findings (Fig. 6).

While most of the results published in the literature refer to single- or short-duration inhalation of relatively high dust concentrations -- in the case of Friedberg it is, for instance, 180 mg/m^3 -- we were interested during the last period mainly in the more prolonged inhalation of industrial-hygienically more important concentrations.

In the following experimental series we made rats inhale daily for 4 hours Dorentruper quartz in an average concentration of 8 mg/m^3. The gravimetric measurements were carried out with a Sartorius electrostatic dust precipitator. The number of particles, determined by means of the

Sartorius Conimeter, was, on an average, 1,200 particles under 5 μ/cm^3. As we were able to repeatedly ascertain on newly entered control animals, the 24-hour retention amounted, with this experimental arrangement, to a reasonably constant 35 gammas per inhalation period.

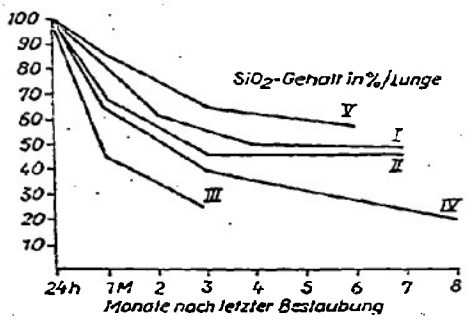

Fig. 6. Elimination Curves of Quartz (V) and Quartz Glass (III) After Short-Term Inhalation at Comparable 24-Hour Retention Values. Quartz glass is separated significantly faster.

I	Argillite	100% = 0.77 mg
II	Argillite	100% = 0.28 mg
III	Quartz Glass	100% = 0.76 mg
IV	Dorentruper Quartz	100% = 0.19 mg
V	Dorentruper Quartz	100% = 0.64 mg

1 -- SiO_2 Content in % per Lung; 2 -- Months After Last Dust Inhalation.

The next table (Table 3) shows the course of such an experiment with 60 inhalations in 90 days and up to 3 months after termination of the inhalation. We have listed the found lung values next to the calculated ones, and have included, besides, the values of the mediastinal lymph nodes. The table shows unequivocally that the purification of the alveolar region is most unsatisfactory when very low quartz concentrations are inhaled over a long period of time. When one takes into consideration the differences shown in the table, one must come to the conclusion that only about 50% of the eliminated dust is separated through the bronchi. The other 50% is deposited in the extrapulmonary lymph nodes. One month after termination of the inhalation, 60% of the dust eliminated in this period is found in the mediastinal lymph nodes, and 100% after 3 months. Thus, in this case, the purification of the alveolar region took place increasingly, and at the end exclusively, through the lymphatic route.

TABLE 3

Dust Absorption and Separation During and After Long-Duration Low-Dosage Inhalation of Quartz (Average particle concentration 1,200 per cm^3) at a Daily Retention of about 35 gammas.

Bestaubung Zahl	Zeit	SiO_2-Gehalt in mg Lungen ber.	Lungen gef.	D*	Drüsen	Dr. + Lu.	D*
8		0,290	0,316	+ 0,026	0	0,316	+ 0,026
16		0,560	0,513	— 0,047	0,004	0,517	— 0,043
24	1 Mon.	0,840	0,799	— 0,041	0,007	0,806	— 0,034
32		1,120	0,973	— 0,147	0,040	1,013	— 0,107
40	2 Mon.	1,400	1,200	— 0,200	0,100	1,300	— 0,100
52		1,820	1,480	— 0,340	0,168	1,648	— 0,172
60	3 Mon.	2,100	1,700	— 0,400	0,190	1,890	— 0,210
n. letzt. Bestaub.	1 Mon.		1,510	11% elimin.	0,315		
	3 Mon.		1,290	24% elimin.	0,536		

eliminiert in 1 Mon. 0,190 mg, davon 0,125 mg in den Drüsen = 66%
eliminiert in 2—3 Mon. 0,220 mg, davon 0,221 mg in den Drüsen = 100%

1 -- Dust Inhalations; 2 -- Number; 3 -- Time; 4 -- SiO_2 Content in mg; 5 -- Lungs; 6 -- Calculated; 7 -- Found; 8 -- Glands; 9 -- Glands + Lungs; 10 -- Month; 11 -- After Last Inhalation; 12 -- Eliminated in 1 month 0.190 mg of which 0.125 mg in the glands = 66%; 13 -- Eliminated in 2-3 months 0.220 mg of which 0.221 mg in the glands = 100%.

Fig. 7 shows a similar experiment in a different presentation. The rats have inhaled dust for 49 days, for 4 hours each day, and have retained on an average 850 gammas SiO_2. After termination of the inhalation, the course of further elimination is excellent. The broken top line shows the sum of the analytical values of lungs and mediastinal lymph nodes. The lower line indicates the lung values (I). It may be seen from the upper line that the sum of the SiO_2 values of lungs and lymph nodes remains approximately the same, while the lung values slowly decrease. Thus, in this case, the elimination from the lungs takes place exclusively through the lymphatic route. The rate of elimination is very low. In the meanwhile we have obtained in this experiment the 8-month value. After 8 months, over 75% of the amount of dust deposited at the end of inhalation (850 gammas) is still in the lungs. The amount eliminated is detectable up to 100% in the mediastinal lymph nodes.

In Fig. 7 the elimination curve of an experiment with short-period high-dosage inhalation of quartz is drawn in

(II) for comparative purposes. After a relatively high rate of elimination in the first month, the additional quartz elimination takes place slowly up to the sixth month, and in fact -- as indicated by the broken line -- mainly through the lymphatic route. From this experiment, too, one can recognize once more the great importance of the lymphatic transport in the elimination of quartz.

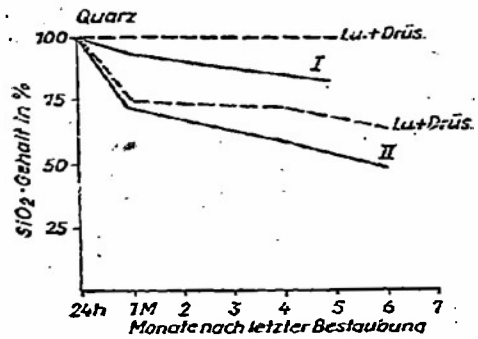

Fig. 7. Elimination Curve after Prolonged Low-Dosage Inhalation of Quartz (I) and after Short-Duration Inhalation (III). The broken lines indicate the SiO_2 content of lymph nodes and lungs; the solid lines indicate the SiO_2 content of the Lungs. SiO_2 content in % per lung.

I = 49 Dust Inhalations 100% = 0.85 mg
II = 6 Dust Inhalations 100% = 1.45 mg

1 -- Quartz; 2 -- SiO_2 Content in %; 3 -- Lung + Gland; 4 -- Months after Last Dust Inhalation.

At the present time we are continuing the experiments with low-dosage, long-duration inhalations on a larger scale. They are thought to be an important foundation for planned inhalation experiments conforming to practice. One must keep in mind that the practical dust concentrations today are everywhere much lower than they were 10-20 years ago.

The results obtained so far appear to us very important. Some authors [10, 13, 18, 32] have come to the opinion on the basis of short-duration inhalation experiments and relatively short periods of observation that the lymphogenic removal of dust is without significance, and that it only plays a role after the breakdown of bronchiogenic elimination. We must, on the contrary, state that in the case of more prolonged and very low-dosed dust stress, as happens in

industrial practice, the purification of the alveolar region from quartz takes place to a large extent and after the termination of the inhalation almost exclusively over the lymphatic route. The bronchiogenic elimination practically ceases after a certain time. Unfortunately this indicates that the quartz dust remaining in the lungs has then penetrated into the tissues on an almost quantitative scale. In the tissues and the lymph nodes, the dust nevertheless acts pathogenically, due to its fibroplastic effect. Thus, the lymphogenic elimination is in no way only a welcome purification process, but at the same time a partial factor in the pathogeneity of quartz. We do not at this time know why Al_2O_3 and TiO_2 hardly get into the lymph nodes.

To this one can add in a summarizing manner that the lymphatic elimination is not something like a secondary process which only plays a role when the bronchiogenic purification has broken down, but rather it is a basic physiological process running parallel to the latter, useful in the resorption of exudates and substances capable of decomposition but unfortunately harmful in the resorption of pathogenic dusts. In fact, the lung tissue is fundamentally endangered by this physiological mechanism.

Today, most authors assume that the dust particles in the free state penetrate into the lung tissues; according to Macklin [6, 7] and Gross [8], they do so probably in the region of the so-called "sumps" [?] together with the excess alveolar fluid. When, however, the alveolar phagocytes do not wander into the interstitia, then the intra-alveolar phagocytosis is of decisive importance for the bronchiogenic purification of the alveolar region. We are, therefore, currently extending our elimination studies to the counting of free alveolar cells, by basing ourselves on the experiments of LaBelle and Brieger [28, 29]. The first results are already in. After a short-term inhalation of aerosil, the cell count jumps to a very high value, but then again drops with the first week of inhalation to one-half. We attribute this to the fact that in a short time, large numbers of alveolar phagocytes were destroyed due to the high toxicity of the aerosil dust. In some cases we could observe in the second week another strong rise in the cell count. This could correspond to a renewed cellular desquamation with secondary phagocytosis of the dust liberated from the disintegrated cell and of the cell fragments.

After a short-term high-dosage quartz inhalation (about 60 mg/m^3), the alveolar cell count rose until three weeks after inhalation, but not so strongly by far as after

aerosil inhalation, and then again drops slowly. The investigations are still in progress and we shall further report on them shortly.

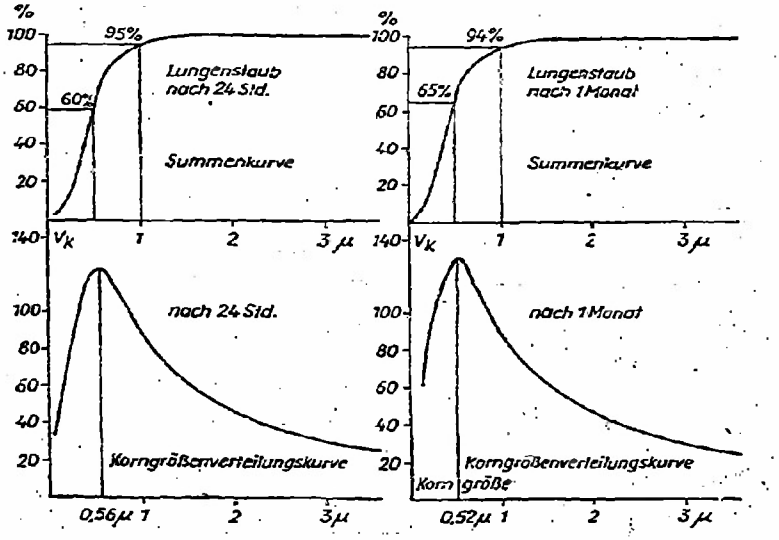

Fig. 8. Particle-Size Distribution of Inhalded Quartz Dust in Rat Lungs 24 Hours and One Month After Inhalation.

1 -- Dust in Lungs after 24 Hours; 2 -- Dust in Lungs after One Month; 3 -- Summary Curve; 4 -- After 24 Hours; 5 -- After One Month; 6 -- Particle-Size Distribution Curve; 7 -- Partiele Size.

In conclusion, we would like to report on current experiments relating to the particle-size distribution of the inhaled quartz dust in rat lungs. After inhalation, the lungs are decomposed with formamide (Thomas and Stegemann) or incinerated according to Nagelschmidt. The quartz dust thus recovered was subjected to electron-microscopic photography. The particle-size measurements were carried out and from the obtained values particle-distribution curves and summary curves were prepared. The last figure in this paper [Fig. 8] shows the particle-size distribution curves and summary curves of the quartz dust retained, respectively, 24 hours and 4 weeks after inhalation. It can be seen that 95% of the deposited resp. retained quartz particles have a size up to 1 µ. The curves determined after 4 weeks exhibit a slightly different course (Fig. 8). The data after a longer exposure period have not yet been interpreted so that an unequivocal statement is not yet

possible. We hope to obtain from this type of experiments information as to whether it is the larger or smaller particles that are better retained resp. eliminated. This question is of great practical importance.

Our report deals almost exclusively with animal-experimental results. It is repeatedly asked in a critical sense whether such results can be transferred to the conditions present in humans. Since the purification mechanisms and pathways are basically alike in man and animal (rat), certain conclusions may indeed be drawn. Nagelschmidt [30] has recently pointed out in Paris that the particle-size distribution of retained dust is similar in rats and humans. We are able to confirm this.

Nagelschmidt has further calculated that also the percentages of retention are comparable in rats and humans: in humans he calculated 2.5-24%, in rats 15-22%. We could calculate in low-dosage inhalation experiments a 24-hour retention of about 20%, so that also here we agree with Nagelschmidt. A certain comparability of the results in rats with the conditions in humans is consoling, and gives rise to the hope that the animal experiments of the future may furnish data which would be extrapolable to humans. Finally, we consider the elimination studies as the foundation of the determination of limiting dust values. Also, one should attempt to learn whether the pulmonary purification can be favorably influenced and what factors are capable of disrupting it.

Summary

The present report deals with quantitative investigations regarding the elimination of inhaled dusts in rats. We pointed out the significance of these studies from the point of view of pneumoconiosis research, industrial hygiene and industrial toxicology in the framework of an introductory literature survey. The elimination of dust depends on the intensity and duration of exposure. The exposure to dust is, in turn, determined by the industrial processes leading to the dust stress: whirling up, mechanical attrition process and melting resp. combustion processes.

In pulmonary purification two phases are to be distinguished, the quick phase -- purification of the bronchi and bronchioli lined with ciliated epithelium -- and the slow phase -- purification of the alveolar region.

The slow phase of dust elimination is dependent not only on the amount of dust absorbed but also on the type of dust. In our investigation at comparable particle sizes, particles with a greater solution velocity also exhibited a higher elimination rate.

The elimination of difficultly soluble dusts extends over months. Here the bronchial route and the pulmonary lymph route may be engaged to differing extents. According to our results, it seems that quartz, above all, possesses a high affinity to the lymphatic system.

We have reported, for the first time, on experiments in which the dust eliminated after an exposure of several months was investigated with dust concentrations near those encountered in practice. Here the lymphotropism of quartz is particularly marked.

The retention and particle-size distribution in the lungs appear to be comparable in rats and humans.

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Translation No. 860

EXTERNAL MEASUREMENT OF THE PULMONARY PRECIPITATION OF RADIOACTIVE GOLD AEROSOL IN RABBITS

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(Director: Prof Dr L Friberg)

Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; pp 75-78

In investigations published up to now the pulmonary deparation has been determined chemically or radiologically on the dead animal. An exception is the work of Albert and Arnet, who have studied the pulmonary separation in humans by means of external measurements, following the inhalation of radioactive iron.

We have investigated the pulmonary precipitation [or separation] in rabbits after inhalation of an aerosol containing radioactive gold. We have chosen gold because of its suitable chemical and radiological properties.

The rabbits inhaled said gold aerosol through a tracheal tube. Afterwards the activity was repeatedly measured from the outside by means of a movable scintillation counter. The aerosol particles which consisted of gelatin with imbedded colloidal particles had a diameter of about 1.5 µ. Fig. 1 shows the particle sizes.

The experimental arrangement for the measurement of pulmonary precipitation is represented in Fig. 2. The results of the experiments are shown in Fig. 3, in the form of a few typical profile curves at different periods after the end of exposure. By planimetry of the profile curves as well as by correcting for the physical disintegration, data could be obtained for the pulmonary precipitation. Fig. 4 describes the pulmonary precipitation. It was found that the half life varies. After 24 hours, the activity had decreased to about 50% in three rabbits and to about 75% in five rabbits.

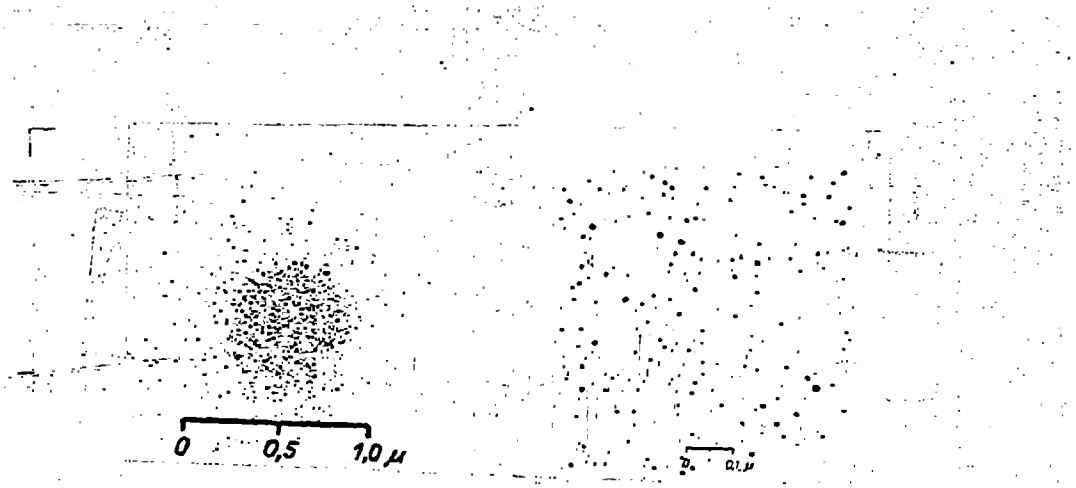

Fig. 1. In the left section of the figure may be seen a gelatine particle containing colloidal gold visible in the very small black dots. -- In the right section, the colloidal gold particles are seen better separated from one another due to the higher magnification.

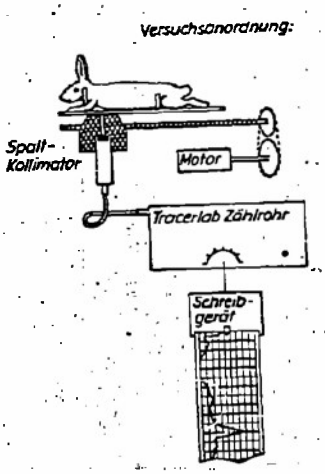

Fig. 2. The rabbit lies on a plate under which a constant-velocity detector is moving. A writing device records the activity distribution measured on the animal.

1 -- Experimental Setup; 2 -- Slit Collimator; 3 -- Tracerlab Counting Tube; 4 -- Writing Device.

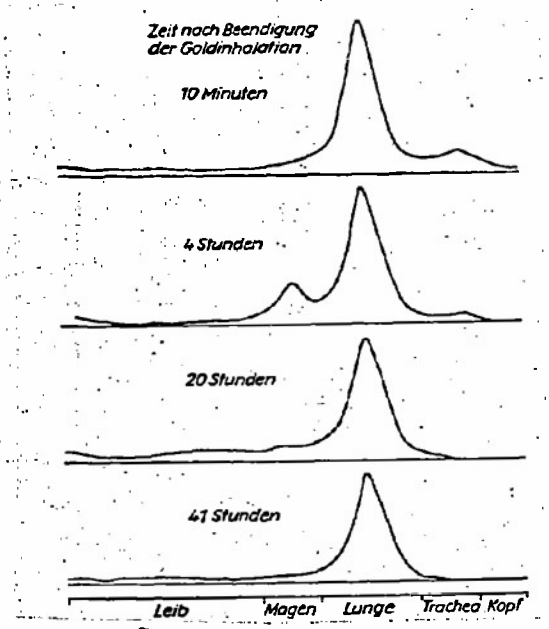

Fig. 3. Curves showing typical activity distribution in head, trachea, lung, stomach and abdomen (half-life compensated). They were registered 10 minutes, 4, 20 and 41 hours after termination of the inhalation of gold.

1 -- Time After Termination of Gold Inhalation; 2 -- Minutes; 3 -- Hours; 4 -- Abdomen; 5 -- Stomach; 6 -- Lung; 7 -- Trachea; 8 -- Head.

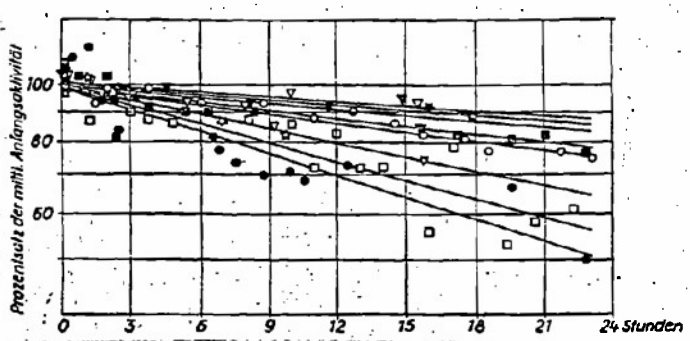

Fig. 4. Pulmonary Precipitation in 8 Animals.

1 -- Percent. of Average Initial Activity; 2 -- Hours.

Repeat analyses in organs such as liver, kidneys, spleen, blood and urine have shown that the resorption from the lungs to blood is less than 0.1% of the amount of the gold deposited in the lungs.

In 28 animals we obtained a measurement of the pulmonary precipitation by means of the method described by us and at the same time performed a measurement of activity in isolated organs, in order to compare our method with a more conventional one.

Fig. 5 shows a good agreement between the two methods.

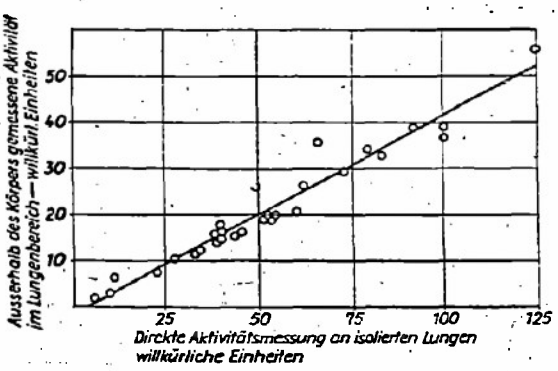

Fig. 5. Correlation of the method of external measurement of the pulmonary precipitation of radioactive gold (Au-198) in living rabbits, described by us, with the well-known activity measurement on isolated lung.

1 -- Activity in the Pulmonary Region Measured outside the Body -- Arbitrary Units; 2 -- Direct Activity Measurement on Isolated Lung -- Arbitrary Units.

We anticipate using this method for the observation of pulmonary precipitation after exposure to different irritating substances. The essential advantages of the method are that, (a) the same animal can be used to obtain several data, and (b) as a result of the half-life of gold, only 64 hours, it is possible to subject an animal to repeat experiments. Hence, this method for the study of pulmonary precipitation requires only a quite small number of experimental animals.

THE INFLUENCE OF DIFFUSING FACTORS ON THE ABSORPTION OF d-CYCLOSERINE ADMINISTERED TO GUINEA PIGS IN AN AEROSOL FORM

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Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; pp 79-87

The employment of certain antibiotics in the form of an aerosol for the treatment of pulmonary tuberculosis has been resorted to in certain cases. The addition of diffusing factors to these solutions which make such aerosols possible has been recommended by Dumon [3]. The expediency of this measure has been confirmed by good clinical results.

We have investigated the effectiveness of a new diffusing agent, Thiomucase, on the penetrating power of d-cycloserine used in the guinea pig as an aerosol. We have selected this antibiotic because it had been used in Dr Dumon's department and because its content in the tissues and fluids of the body is established relatively easily and speedily. In order to compare thiomucase (which acts on the chondroitine sulfuric acid) with another diffusing factor, we have made use of hyaluronidase (acting on hyaluronic acid).

Our intention was to study the following questions:

1) Does the repeated use of d-cycloserine as aerosol lead to an accumulation of this medicament in the pulmonary parenchyma, and do the diffusing factors increase this accumulation?

2) What are the differences between the two diffusing factors?

3) What is the smallest amount of diffusing factor with which a higher penetration is achieved than is done by the cycloserine aerosol alone?

Experimental Method

1) Analytical Methods. Cycloserine was determined in the blood, urine and bile according to the method of L R Jones [4]. For the determination in the tissues (lung and kidney), we have somewhat modified the method given by Coletsos et al [2]. We place the tissue into two parts by weight of distilled water in order to suspend it by the expedient of dividing it into fine particles by means of an Ultra-Turax. The homogeneous paste thus obtained is kept for 24 hours in the refrigerator at +4°C with intermittent shaking. The fine constituents are centrifuged off and the cycloserine content in an amount corresponding to the centrifugate determined by Jones' method.

2) Methods of Application of Cycloserine. The guinea pigs were divided into four groups.

Group I obtained 5 mg cycloserine per kilogram weight for three days through an esophagus tube. The animals were killed one hour after the last feeding.

Groups II-IV obtained cycloserine aerosols alone, or with diffusing factors. In Group II, it was cycloserine alone; initial solution 5 g% cm^3, one hour per day. In Group III, the same plus two 100-unit ampoules of thiomucase per 20 cc solution. In Group IV, the same as in Group II plus two 250-unit ampoules of hyaluronidase per 20 cc solution.

3) Duration of Treatment. For the testing of the accumulation, the duration of the treatment was varied from 5 to 22 days. For comparison of the two diffusing factors, a single aerosol session, only, was employed. For the determination of the minimum dose of the diffusing factors, too, only a single aerosol session was resorted to. The animals were killed 15 to 30 minutes after the termination of the session. The urine was obtained by puncturing the bladder. In male animals, we attempted to obtain the entire amount of urine by tying up the urethra during the last aerosol session.

4) Determination of the Minimal Dose. For the preparation of the aerosols, a 5 g% cycloserine solution was used; 20 cc of this solution was treated with 2, 1, 1/2 or 1/4 ampoule diffusing factor.

Results

1) Cycloserine "per os." The table shown below presents the average results from four animals:

TABLE 1

Cycloserine per os. Results in microgram cycloserine per cc or gram of tissue

Blut	Lunge	Urin	Ausscheidung je Stunde
2	19 -	31	50

1 -- Blood; 2 -- Lung; 3 -- Urine; 4 -- Separation per Hour.

The last column shows the total amount of cycloserine in micrograms eliminated in about one hour.

2) Accumulation. Table 2 lists the results after a treatment of 5, 15 and 22 days, respectively. In all cases, average values of at least three animals are given. All guinea pigs had the same weight.

TABLE 2

Accumulation Experiment

Behandlungsdauer in Tagen	Cycloserin allein			Cycloserin + Thiomukase			Cycloserin + Hyaluronidase		
	Lunge	Blut	Urin	Lunge	Blut	Urin	Lunge	Blut	Urin
5	12	6	—	9	6	52	14	8	—
15	10	3	38	6	6	280	7	3	56
22	15	5	47	6	4	35	3,5	0	58

1 -- Duration of Treatment in Days; 2 -- Cycloserine Alone; 3 -- Lung; 4 -- Blood; 5 -- Urine; 6 -- Cycloserine + Thiomucase; 7 -- Cycloserine + Hyaluronidase; 8 -- Results in micrograms per g or cc.

From these experiments it follows that prolonged treatment does not cause an accumulation of the antibiotic in the pulmonary parenchyma, regardless of whether it is administered with or without diffusing factor. It seems that the concentrations in the urine are higher when diffusing factors are used. This has led us to investigate what effects these factors have on the total elimination of cycloserine over a given period of time.

3) Comparison of the Two Diffusing Factors. Since the previous experiment has shown that the cycloserine concentration was increased in the urine in cases where the aerosols had been given with diffusing factors, we believed that this could be used as a means for the evaluation of the effectiveness of these factors. In Table 3, we give the results of different series of experiments which comprised each time at least four guinea pigs.

TABLE 3

Comparison of the Two Diffusing Factors

Aerosol	Lunge	Niere	Blut	Entleerter Urin in ccm	g/ccm im Urin	Gesamtausscheidung
Cycloserin allein	2	4	2	4	34	136
Cycloserin + Thiomukase	2	3	0,5	4,6	45	207
Cycloserin + Hyaluronidase	3	1	0,25	3,7	30	110

1 -- Lung; 2 -- Kidney; 3 -- Blood; 4 -- Urine Excreted in cc; 5 -- g/cc in urine; 6 -- Total Elimination; 7 -- Cycloserine alone; 8 -- Cycloserine + Thiomucase; 9 -- Cycloserine + Hyaluronidase.

The results are likewise expressed in micrograms per gram tissue or cc urine, as well as in micrograms of cycloserine separated over a given period of time, generally 1 1/2 hours, reckoned from the beginning of the aerosol session. For the sake of comparison, we also give the values for cycloserine without the addition of a diffusing factor.

The results are given in micrograms of cycloserine per g of tissue or cc blood resp. urine. In the last column, the results are shown in micrograms of cycloserine.

If one assumes, along with Anderson [1], that cycloserine is quickly eliminated from the body, then the excretion in the urine can be interpreted as follows:

The cycloserine which has entered the greater circulation is quickly excreted in the urine. The amounts of medicament which are found in the urine are in direct proportion to the amount of cycloserine that has gotten as far as the renal region.

4) Determination of the Minimal Dose. In the following tables we show the results obtained with decreasing amounts of diffusing factors and equal amounts of cycloserine. Each series of results gives the average of the

values from three guinea pigs. In addition, we entered the values for the bile. In the latter case the values are given in micrograms per cc bile. The average liquid quantities obtained by puncturing the gall bladder of the killed animal amounted to 0.7 cc.

TABLE 4

Determination of the Minimum Dose (Thiomucase)

Einheiten Thiomukase auf 20 ccm	Lunge	Niere	Blut	Galle	Urin	Ausscheidung im Urin
200	20	5	4,5	38	40	200
100	16	4	3	16	25	174
50	12	5	3	37	26	180
25	6	0	2	5	24,5	173

1 -- Thiomucase Units per 20 cc; 2 -- Lung; 3 -- Kidney; 4 -- Blood; 5 -- Bile; 6 -- Urine; 7 -- Elimination in the Urine.

The results are expressed in micrograms cycloserine per g tissue or cc liquid. In the last column the results are expressed in micrograms cycloserine.

TABLE 5

Determination of the Minimum Dose (Hyaluronidase)

Einheiten Hyaluronidase auf 20 ccm	Lunge	Niere	Blut	Galle	Urin	Ausscheidung im Urin
500	29	6	4	38	29	116
250	11	5	3	20	26	128
125	9	5	3	16	27	81
62,5	5	0	1	3	22	85

1 -- Hyaluronidase Units per 20 cc; 2 -- Lung; 3 -- Kidney; 4 -- Blood; 5 -- Bile; 6 -- Urine; 7 -- Elimination in the Urine.

The results are expressed in micrograms cycloserine per g tissue or cc liquid. In the last column the results are given in micrograms cycloserine.

From this series of experiments, it is seen that one can use thiomucase in smaller doses than was originally employed; thus the minimal dose amounts to 50 units per 20 cc,

that is, 2.5 units per cc. In the case of hyaluronidase, however, it seems that one cannot go below 12.5 units per cc.

Summary

From the experiments which we have carried out, it follows that

- The use of cycloserine as an aerosol in guinea pigs does not lead to an enrichment of this medium in the lung parenchyma; the addition of diffusing factors does not promote enrichment;

- Thiomucase is the better diffusing factor, when one uses as a test of the penetration of the medicament in the organism the amount of eliminated substance found in the urine after an aerosol use of one hour;

- The minimal dose of thiomucase amounts to a half ampoule or 50 units per 20 cc cycloserine solution (2.5 units per cc).

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Discussion

Siegert, Berlin:

Mr Klosterkotter has spoken, among other things, on the purifying action of the nose of the animal in regard to the dust content of the inhaled air. In different animal species different degrees of purification were found. This allows one to conjecture whether in man a different purification function of the nose does not activate different degrees of dust stress in the lungs? For this we do have certain points of reference. First of all, in the case of man two nasal stenoses are present, of which the front one has been called a "nose lid" by Mink. Semirak has carried out pneumotachographic investigations of the nasal ventilation and ascertained that asthmatics have a relatively lower resistance in the nose. Our own investigations indicate that the asthmatic -- and I am not at first going to decide whether it is a primary or secondary asthma -- does not or cannot use the frontal nasal stenosis. Since, for this reason, most likely, in different persons a different grade of purification effect of the inhaled air is attained by means of the nasal stenoses, I would like to ask whether the influence of individually varying nasal stenoses on the degree of silicosis had been investigated, since it has been ascertained that despite equal exposure the degree of silicosis is different.

Neumann, Wurzburg:

In reference to Mr Oldiges' interesting report, I would like to ask a specific question relating to the inhalation and absorption of methylene-blue aerosol. The amount of methylene-blue aerosol supplied was compared with the portion recovered in the trachea and lung. I do not, unfortunately, remember whether the exhaled amount is determined analytically. Only in that case can one make a significant statement regarding the total amount absorbed resp. the portion of the aerosol taken up. In addition, it was said that no methylene blue had been found in the urine of the animals. This, however, does not mean that none had been resorbed. And I am interested particularly in the eventual resorption of the methylene blue contained in the aerosol. This is somewhat related to the lecture of Prof Pastor who -- if I could follow the figures to some extent -- mentioned a considerable resorption of d-cycloserine.

Leers, Leipzig:

I have two questions in regard to the lecture of Mr Oldiges. The first question concerns the method of measuring the aerosol. An average particle size of 0.6 µ was given. How was this measured and how did one avoid a change in this particle distribution through evaporation or condensation phenomena, considering that one dealt with an aqueous solution that was atomized?

The second question concerns the following: In the lecture mention was made of the Findeisen theory resp. its extension by Landahl etc. There the conditions in man are taken into account under quite different presuppositions. How far can one transfer these conditions to animals and to what extent has it ever been attempted to calculate these things for rats or other animals under the same presuppositions as were made by Findeisen for man and to compare these calculated figures with those observed experimentally. One would then perhaps obtain also a certain pointer whether it is really true, as was stated in the lecture and as I have understood it, that the breathing rate has a decisive influence on the deposition, or whether it is not rather the anatomical conditions and the volume rate of breathing that are of decisive importance for these problems.

Bisa, Grafschaft:

Perhaps I can already answer some of the questions raised so far.

First of all, the question of Prof Neumann, concerning the amount of exhaled dust in relation to the inhaled amount. It is extremely difficult to measure the exhaled amount during an experiment lasting several hours. The valve-clack mechanism of a respiratory valve offers extraordinary technical difficulties and represents an additional source of error for the measurement proper. The moment we use a valve, be it as fine as possible, and formed of cilia, we disturb the free aerosol stream. Our biochemists could establish, however, that no leucobase of the methylene blue had deposited in any form whatsoever in any other tissue outside the respiratory tract. Nor could leucobase be detected by differential and difficult methods.

Now, the question of aerosol measurement. For measuring particles of 0.6 µ, we not only use conventional methods but also a new device developed in our institute. Within a few minutes, say 10 minutes, there is obtained via the

photomultiplier an aerosol spectrum that is measured automatically. Through feedback it makes it possible for the aerosol milieu to remain relatively constant.

The last question of Mr Leers. The calculations of Findeisen essentially have as object of discussion -- as the other authors have also given -- particles lying in the so-called lung-penetrable region of 0.5 to 3, 4, and 5 μ. On the basis of thorough experiments, however, we have become convinced that the particles under 0.5 μ diameter -- that is, where our normal measuring methods have so far been withdrawn -- are of extraordinary biological importance, and that we cannot neglect this fraction. This is the reason also for our investigation at a particle-size spectrum whose particle-size maximum is 0.6 μ, a spectrum which otherwise is relatively unusual. The customary atomizers used for therapeutic purposes are fixed for a particle size of about 1 μ. Despite this, we would not like to neglect this fraction which is known to lie below that. On the other hand, we will not want to establish any relationships with the old Findeisen concepts, since this is simply not possible. This is merely a pure experimental material and on the other side we have very artificial theoretical calculations.

Friedberg, Gottingen:

I'd like to ask a few questions. First of all of Mr Oldiges. One of the fundamental points of his data was the measurement of the volume rate of respiration, if I understood him correctly. He gave the percentual portion of methylene blue that has remained in the respiratory tract in function of the inhaled amount. It seemed to me that he had obtained a very low value for the volume or size of breath of the rats. Incidentally, I happen to know the values of the volume of air breathed per minute by rats as discussed in the literature. They fluctuate between 100 and 300 ml per minute. And here figures like 24 ml are given. When we compare this with the data given so far, it would still be very interesting to learn how this volume rate of breathing has been measured and how he came to this small value, since when one substitutes the other minute volumes then all data would have to be multiplied or divided by a factor of at least 5.

In regard to the very interesting and extensive investigation of Mr Klosterkotter, I naturally cannot take, alone, any position. This was a many-sided and multi-faceted material. But perhaps I will be allowed to ask a question regarding two matters that interest me very much: 1) How does the particle size influence the slow phase of dust elimination?

Have you also carried out investigations with a qualitatively identical material, and by qualitative I mean both from the chemical and the mineralogical-crystallographic point of view? Have you any indication that in the case of a qualitatively identical material the elimination takes place to the same extent for small particles as for large particles? 2) It is a disconcerting finding that at a very low dust concentration of the inhaled air the dust elimination practically ceases. Have these investigations been carried out so far only for quartz and does this hold true so far only for quartz or also, under certain conditions, for TiO_2 and Al_2O_3 as well?

Oldiges, Grafschaft (Concluding Remarks):

I assume that the first questions of Dr Bisa have already been fully answered, and therefore I will concentrate on the last question. Mr Friedberg asks about our method of measuring the respiratory volume. In the tables I have only given the amount of inhaled air. We have fully separated the inhaled and exhaled air. Thus, it is a matter of 24 cc inspired air per minute. We have measured this by means of an inclined manometer, and, as was shown on the figure, we have striven toward a complete leak-tightness of the head of the experimental animals connected to the device. And when one attains such a full leak-tightness one will arrive at different respiratory volumes than have so far been given in the literature. The values which we have measured fluctuated relatively strongly. This depends on the excitation state of the animal. But the values communicated here, that is, taken twice, 48 cc/min are surely values that are being widely discussed.

Klosterkotter, Munster (Concluding Remarks)

I would first of all like to take a position with respect to the question of Mr Siegert. It is an old problem -- which was not discussed here -- whether it is the nasal breathers or oral breathers that are more likely to develop a silicosis. This question is not yet cleared up with certainty. Earlier, Prof Lehmann had been much occupied with the subject but was not able to formulate a final statement on it. Most likely we cannot explain the differential extent of a silicosis by this factor, since we know when we recover dust from the lungs and analyze it that it is possible for a marked silicosis to exist in the patient already with a deposition of 10 or 20 g dust in the lungs while another patient will remain unaffected. Also, even with the same amount of deposited dust there exist quite considerable

differences in the extent of silicosis, and hence we cannot in this regard introduce at the moment an additional factor -- effectiveness or non-effectiveness of the nasal stenosis -- as long as we do not yet know how it happens that there exist in the presence of the same deposition different grades of silicosis. In this matter, constitutional factors play a role about which we know very little as yet. It is interesting, moreover, that it is always repeatedly stated that persons with asthmoid constitution and persons with asthmatic reactions develop silicosis less readily than normal healthy persons.

The question has also been raised and left unanswered whether the breathing rate has any influence on the deposition. This we ourselves have not determined, but there is an excellent new study by Brown in the US who is indeed one of the most important specialists in this area. He has reported on the subject at Oxford. Brown, in an experiment carried out on his person, has inhaled bacillus spores of 1 μ, at different breathing rates and different lengths of breath-holding. It was found that a considerable dependence exists on the breathing rate and length of breath-holding. The smaller the frequency and the longer the breath-holding period (the latter is naturally in direct relation to the former) the greater the deposition. This can naturally be determined better in a human self-experiment than in an animal.

Now, to Mr Friedberg. So far we have performed these experiments with the dusts which I listed in the first table. Our most important comparative investigations are those between aerosil and aluminum oxide, both being dusts of the same particle size, dusts of a comparable surface area. Here the separation takes place quickly in the case of aerosil which is relatively readily soluble, and very slowly with aluminum oxide that is difficultly soluble. I have not so far had any dust at hand that, while having the same properties, now has large particles and now small ones. I cannot say anything in this regard. If you were able to name such a dust for me, I will gladly enter it immediately in our research program.

We have performed these last investigations with protracted inhalation of low concentrations so far only with quartz. The program is such a large one that for the time being we must continue to work with this substance. Naturally, the program will have to be extended to inert dusts such as TiO_2 or less dangerous ones such as aluminum oxide: this much is quite clear. I would, however, predict already

at this stage that one will hardly be able to assume that these dusts penetrate into the lymph glands -- that is, removed lymphogenically -- to the same extent as in the case with quartz. I refer once more to the statement of the Japanese authors. Silicosis runs its course in the lymphatic tissue or in dependence on the lymphoid tissue, while for example the aluminum-dust disease of the lung occurs in the parenchyma at quite different sites and the glands are little involved. This is an open question which we must clarify. I assume that the heightened irritant effect of quartz dust makes possible its quick penetration into the tissue. It is certain that after about 2-3 months the bronchogenic elimination in these experiments comes to a full stop, and any elimination still taking place will proceed via the lymph vessels. This is a prediction which, incidentally, Prof Giese has already made two years ago in Bochum. He said that at low concentrations most of the dust would be removed via the lymphatic system. He made this statement also in reference to quartz.

Translation No. 862

INVESTIGATIONS ON THE ABSORPTION OF AEROSOLS BY PLANTS

THE INFLUENCE ON PLANTS OF AIR IMPURITIES, PARTICULARLY LIME- AND CEMENT DUST

by Prof Dr A T Czaja

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Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; pp 88-102

Among the dusts emitted in masses of clouds by industrial plants, the greatest danger is offered by those which, upon contacting the wet surfaces and living organisms split off hydroxy ions, that is, exert an alkaline effect:

Lime and Cement Dust (slightly soluble)	Water Glass and Soda Dust (completely soluble)

Of the above, the largest role is played by lime- and cement dust due to the frequency of occurrence of the corresponding industrial plants. These dusts, like the others in this group, are fateful for the plants when they are emitted in larger amounts and come to settle so densely that they may form marked deposits on the plants which may lead to crust formation on the leaves and other organs.

The Origin of Cement Crusts

Practically nothing is known regarding the atmospheric conditions during the aerosol formation (solid aerosol) and the settling of dust. After Kreutz and Walter (1956) cloud formations hinder the rise of dust clouds, and high atmospheric humidity leads to immediate settling of the particles charged with moisture (condensation nuclei), hence an inhibition of the further spreading of the dust cloud. The cement

crusts formed adhere solidly to the surface of the organs (leaves, stems, fruits), in the coniferae around the needles. The cement crusts consist of two layers, an inner one, directly on the leaf surface, and an outer one on top of the former. The inner layer is glassy, crystalline and flat, mirror-like; it follows the shape of the cell surfaces with microscopic accuracy; the external layer, on the contrary, is roughly grainy and terminates at the surrounding air (Figs. 1-3). The inner layer consists of cement bound immediately on the living cells. The external layer consists of cement particles which are hardened in the air by moisture in a grainy way. Perhaps the particles reaching the plant from the cement cloud also carry along hydrate envelopes. So far we do not have any data regarding this matter.

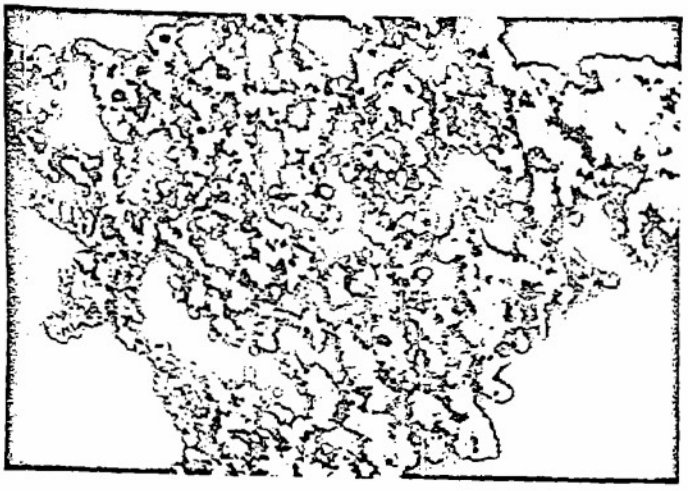

Fig. 1. Cement Crust (Broken-Off Piece) from an Oak Leaf. Top Side. Magnification: 10:1.

The Setting of the Cement

The setting of cement on application of water -- the hydration of cement -- is composed of the hydration of the determinant components of its mineral content. The latter comprises essentially the four components which arise on combustion in the clinkers:

Tricalcium silicate,	Tricalcium aluminate,
Dicalcium silicate,	Tetracalcium aluminate ferrite.

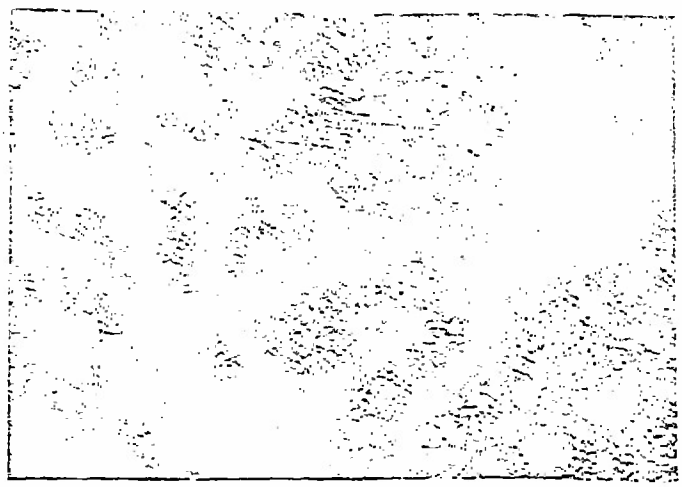

Fig. 2. Cement Crust (Broken-Off Piece) from an Oak Leaf. Bottom Side. Magnification: 16:1.

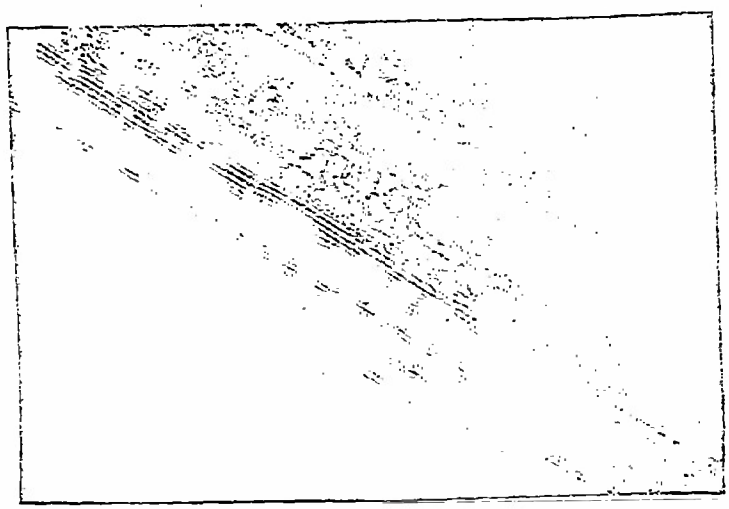

Fig. 3. Cement Crust (Broken-Off Piece) from a Pine Needle. Bottom Side. Magnification: 16:1.

The following equations indicate in a somewhat abbreviated form, for each component, the course of hydration to the extent that this is known. The reaction of the four essential mineral components of the cement clinkers on contact with water proceeds as follows:

Tricalcium silicate

$$2(3CaO \cdot SiO) + 6\,H_2O \longrightarrow \underbrace{3CaO \cdot 2SiO_2 \cdot 3H_2O}_{\text{Tobermorit}} + 3Ca(OH)_2$$

(49 Gewichts-% Kalkhydrat)
(49 Parts by Weight Calcium Hydroxide)

Dicalcium silicate

$$2(2CaO \cdot SiO) + 4\,H_2O \longrightarrow \underbrace{3CaO \cdot 2SiO_2 \cdot 3H_2O}_{\text{Tobermorit}} + 3Ca(OH)_2$$

(21 Gewichts-% Kalkhydrat)
(21 Parts by Weight Calcium Hydroxide)

Tricalcium aluminate

$$3CaO \cdot Al_2O_3 + 6\,H_2O \longrightarrow 3CaO \cdot Al_2O_3 \cdot 6H_2O$$

Tetracalcium aluminate ferrite

$$4CaO \cdot Al_2O_3 \cdot Fe_2O_3 + 2Ca(OH)_2 + 10\,H_2O \longrightarrow 3CaO \cdot Al_2O_3 \cdot 6H_2O + 3CaO \cdot Fe_2O_3 \cdot 6H_2O$$

The hydration of calcium oxide

$$CaO + H_2O \rightleftharpoons Ca(OH)_2$$

The Cause of the Bilayer Character of Cement Crusts

Cement grains whose diameter is greater than 60 μ cannot be hydrated throughout their entire cross section since the gel layer formed on the surface no longer permits the diffusion of water.

In regard to the cement crust this means, however, that the inner layer cannot be thicker than 30 μ. All cement having a higher diameter can be only conglomerated by the atmospheric moisture. It is in this manner that the two-layer structure develops.

The difference between the conditions of the inner crust layer and that of the outer layer consists in that the setting of the cement particles in the inner layer had taken place by means of sufficient water so that a uniform glassy-crystalline layer developed. This hydration process may take place -- below the more or less thick outer cement layer covering it -- only in such a way that the cement particles lying immediately on the epidermis of the affected organs make use, for the purpose of setting, of the transpiration water of the plant organs made available in the epidermic cells themselves, their external wall and cuticles, as well as in the mesophyll cells lying immediately underneath (in the case of nerves also in the collenchyma).

During the last few years numerous fine small

canals running perpendicular to the surface have been found in the external wall of the epidermic cells (Schumacher, 1942; Lamberts, 1954; Franke, 1960, etc.); these canals have been named ectodesms. These are brought into causal relationship with the giving off and absorption of dissolved substances through the epidermic cells.

The external layer of the cement crust consists of particles which are conglomerated with one another through the absorption of moisture from the surrounding air without, however, having undergone a hydration process with the necessary amount of water. As a result, the formation of a uniform, fully set layer has not occurred, and the individual cement grains have to some extent preserved their individuality (Czaja, 1961).

On scraping off the cement crust adhering to leaves and needles, the two layers often separate from one another, and the fragments then lie next to each other (Fig. 4).

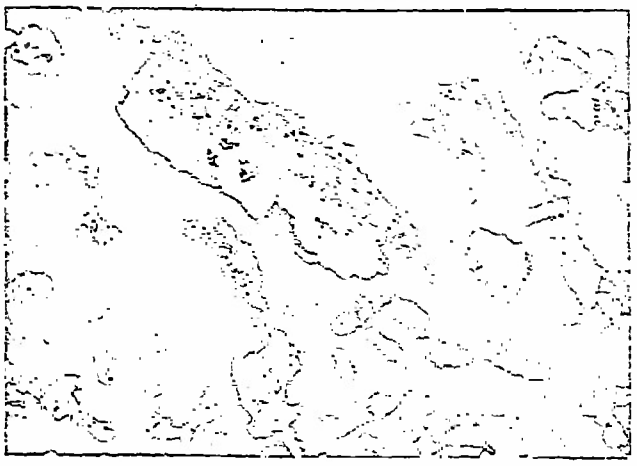

Fig. 4. Cement Crumbs of the Two Layers of the Crust of a Fir Needle; Magnification: 10:1.

Leaves covered in this way with a cement crust, or even fully enveloped plant organs (conifer needles) are surrounded with a firmly adhering stiff crystalline crust which closes up also the stomata (Fig. 5). In this way the gas metabolism through the stomata (stomatic transpiration) is stopped either monolaterally or on all sides, and so is, in a similar manner, the gas metabolism through the cell walls of the epidermis (cuticular transpiration).

Fig. 5. Fir Needle, with Cement Crust Peeled Off, the Stomata Openings Are Still Closed Up by Cement. Magnification: 10:1.

Cement brought into contact with water splits off hydroxy ions, exactly as does lime (white lime, quicklime); the splitting in the case of cement occurring in the setting processes, in the case of lime in the formation of hydrate. The "mixing water," that is, the amount of water wetting the cement, immediately exhibits, exactly as does the "mixing water" of lime, a strong alkaline reaction, pH = 12. Red litmus paper will turn intensely blue and phenolphthalein paper intensely red. Indicator paper will also react in a corresponding manner (Czaja, 1960).

On air the reaction of the cement and lime deposits changes due to the absorption of CO_2. In lime deposits, the reaction drops to a pH of 8 and remains constant. It thus attains the reaction of (precipitated) $CaCO_3$ wetted with water, or of natural, powdered limestone. Cement deposits (crusts) retain a pH of 10 or over a long period of time, but after a prolonged period their pH, too, decreases to 8.

The Action of the Cement Crust on Plant Cells

Under the influence of the hydroxy ions in a highly supersaturated solution, liberated upon the setting of the cement on the epidermis of the plants, there takes place a penetration into the epidermic and mesophyll cells. As a sign of this, one may observe, first of all, the disturbed arrangement of the chloroplasts in the cells; further, and frequently, a change in their appearance and their inner colloidal structure. In the unaffected green leaf cells, the chloroplasts are either round and adhere to the cell

walls in a single layer evenly spread in the cytoplasm, or also in the case of a very large number, the chloroplasts may lie so thick that they mutually flatten out and then assume a polygonal shape. A section through the leaf appears evenly green. Under the influence of the penetrating strongly alkaline solution, the shape of the chloroplasts is altered. They shrink, frequently take on an almond-shaped form. The latter are displaced in the cells and are scattered around more or less irregularly. The section through such a leaf tissue appears lightened, no longer evenly green. The content of the chloroplasts disintegrates into grains (Figs. 6-9). At the same time the cytoplasm also coagulates. Between the chloroplasts, light-refracting plasma skeins of different course become visible which also cause the chloroplasts to be displaced. Large vacuole-like regions in the cell become quite free of cytoplasm.

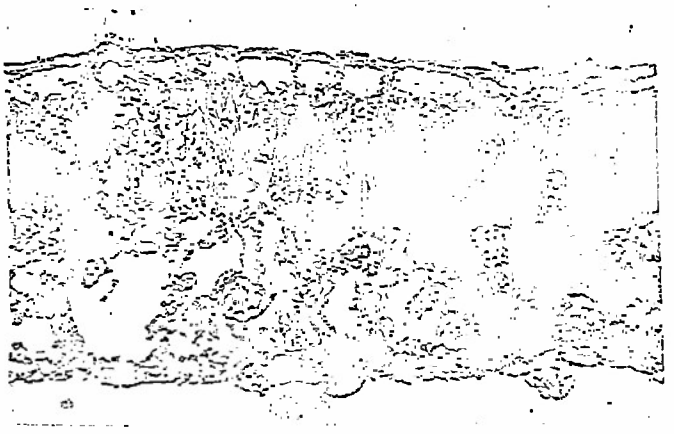

Fig. 6. Oak Leaf, Cross Section, Healthy; Magnification: 100:1.

The latter often strongly coagulates and draws the chloroplasts together into one or even two corners of the mostly stretched cells. This stage is already that of a serious damage in which photosynthesis can no longer take place. Occasionally, one also notes in the neighborhood of the more and more dissolving chloroplasts fragments of starch grains or very small grains of the no longer drained assimilation products. In the green cells of the coniferous needles, a large number of oil droplets appear in the cells. This, too, is a sign of injury. Leaves and other organs damaged in this way exhibit a higher susceptibility to disease-causing agents, for example, fungi (Figs. 10 a and

b). With the destruction of the cell contents, there sets in a saponification of the lipoids of the cytoplasm and the chloroplasts. With this there occurs a discoloration of the chlorophyll and hence also of the leaves.

Fig. 7. Oak Leaf, Cross Section, Injured by Cement; Magnification: 100:1.

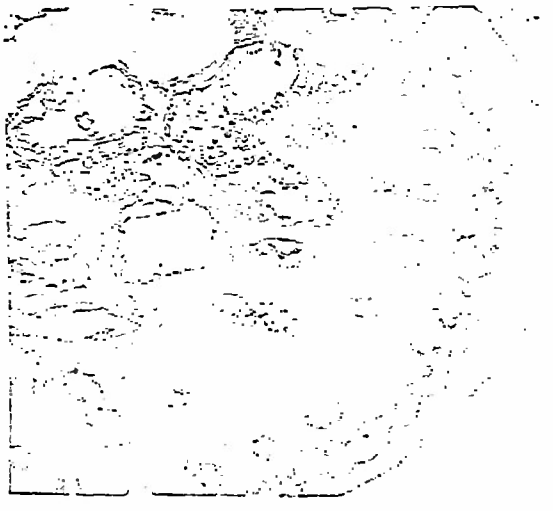

Fig. 8. Fir Needle, Cross Section, Healthy; Magnification: 100:1.

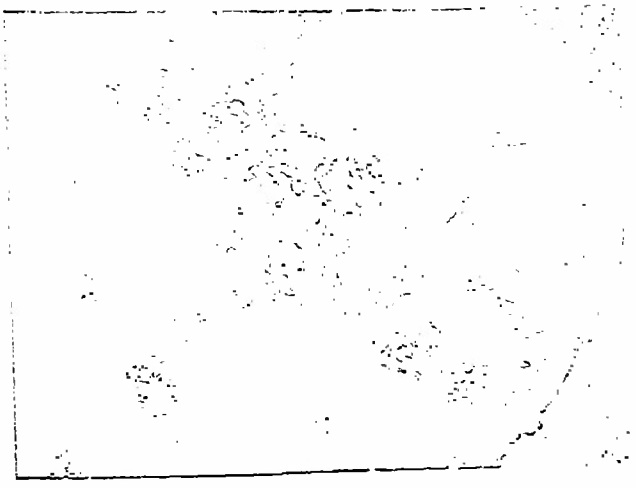

Fig. 9. Fir Needle, Cross Section, Injured by Cement; Magnification: 80:1.

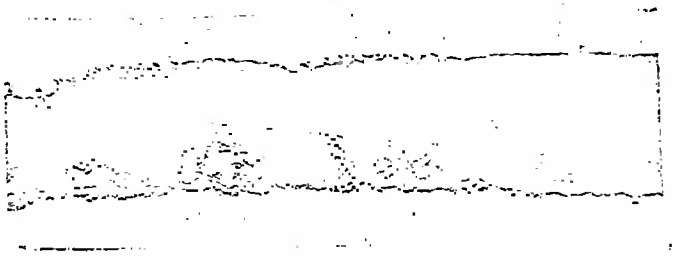

Fig. 10a. Oak Leaf, Cross Section, Healthy; Magnification: 40:1.

In dust-deposition experiments with cement dust for the determination of eventual injury, it has been repeatedly established that no damage occurs in the leaf tissue [e.g., Haselhoff (1919), Lecrenier and Piquer (1956)] even if the yield of the dust-covered plants was much less compared with the unaffected ones. So far, in such dust experiments, the fact has been left out of consideration that the conditions for the formation of cement crusts which result from the settling of a cement dust from an emitted cloud and which at the present time are not adequately detected must be quite different from that resulting from a loose spraying of the leaves with cement dust. In the latter case a true, adherent and two-layer cement crust never develops, nor does the course of the chemical processes of setting (hydration) take

place immediately on the surface of the cells. The latter condition, however, seems to be an indispensable precondition for the onset of serious damages in the plant organs.

Fig. 10b. Oak Leaf, Cross Section, Injured by Cement, after 48 Hours with Marked Fungus Growth on the Under Side. Magnification: 40:1.

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Discussion

Schonbeck, Bochum:

I would like to confirm the interesting presentation of the lecturer also in regard to the subject that we, too, have found regular impression of plant organs with all their individual components, the stomata, epidermic cells, etc. in the cement-dust layers covering them. This has led us to seek leaf impressions suitable for microscopic studies prepared by means of other substances. We found one such substance, the "Uhu" [cement].

We believe to have observed, with a high probability, also the increased susceptibility of plants covered by dust from cement plants. Thus, we noted that sugar-beets treated with such a dust were more susceptible to Ceraspora Beticola (literature report from the State Office for Soil Protection [Landesanstalt fur Bodennutzungsschutz], Bochum).

We were able to note cell changes similar to those shown by the lecturer, under the influence of flying ashes. Thus, in plants naturally contaminated with brown-coal flying ashes we noted a vacuolization of the cell contents,

deformations of the cell nucleus, etc., in cells or leaf-cell layers. Similar observations could occasionally be made in leaves contaminated with flying ashes from coal. The question arises as to whether in this case, too, the cell alterations which resemble very much those brought on by cement dust, are to be attributed to tri- resp. dicalcium silicate resp. the $Ca(OH)_2$ arising therefrom.

In reference to the thickness of the cement layer, I would like to note that the figures given by the lecturer are, in our experience, too low. I am acquainted with cement-dust deposits on trees whose thickness exceeds 5 cm, and leaf deposits amounting in thickness to several times that of the leaf.

v. Eichborn, Neu-Gliching:

Under what conditions did the author find that the cement dust fell off without crust formation? Was only the high concentration of decisive importance in this matter or perhaps also the physical-chemical adhesion conditions?

Funke, Dusseldorf:

I found Prof Czaja's lecture particularly interesting. It is nevertheless necessary to say something fundamental about the emission of dust from cement works so that the audience should not be left with the impression that "clouds of cement dust" go forth from the cement works. The types of dust in a cement work are quite varied in their characteristics and effects. We must distinguish between the raw-material dust consisting of limestone, lime marl, clay, etc.: then the crude-powder dust composed of the above-mentioned substances and consisting of about 80% $CaCO_3$, and finally coal dust, clinker dust and cement dust. The dust which is carried away during the combustion process by kiln flue-gases is called flue-gas dust or cement-kiln dust. It is only this dust that gets into the atmosphere from the chimneys, and is of importance for the generation of clouds in the vicinity of cement works, while all other types of dust, particularly cement itself, are not thrown out unless one includes the small amounts of cement dust which is whirled up on loading and which settles again already in the plant itself. Eighty percent of the cement-kiln dust consists of harmless crude powder which is mixed, according to the combustion process, with varying amounts of clinker dust, coal ash and alkali sulfates. Thus, it is not identical with cement dust and does not harden the cement, so that the cement reactions depicted by Prof Czaja cannot occur.

During the last few years dust-deposit experiments on plants have been performed also by others, among them Prof Scheffer of the University of Gottingen. H. Pajenkamp has reported on these in detail in the periodical Zement-Kalk-Gips (Cement, Lime, Plaster of Paris). It is useful to point out that in the covering with amounts of dust that are possible in the vicinity of some cement works, in no case has any injury to the plant been ascertained. Therefore, I would like to say that the strong crust formation depicted by Prof Czaja must have special causes; it cannot be attributed to the usual cement-kiln dust. It could well be that in the immediate surroundings of older works, particularly when the latter work under unfavorable atmospheric conditions, the dust formations are stronger, yet it would be erroneous to look upon this generally as a measuring stick of the emission damages in the vicinity of cement works.

We would therefore be most grateful if we could discuss in detail with Prof Czaja his research results and problems, perhaps in our own research institute where all the scientists familiar with this problem are present, and accordingly we would like to extend to him our invitation to such a discussion.

Wentzel, Bochum:

After the most interesting studies of Prof Czaja, we must include cement dust -- despite the restricting remarks of Dr Funke -- among the phytotoxic dusts. This is not surprising for one familiar with the effects of atmospheric impurities. In any event, it had been stressed at the last session of the VDI (Verein Deutscher Ingenieure = Society of German Engineers) symposium on "Keeping the Air Pure" (21-23 Sep 60 in Wiesbaden) by the lecturer that cement dust is harmless (cf. Staub (Dust) 21, 2: 91-94 (1961)). This was confirmed in various research studies. This affirmation, however, is a fiction. We are acquainted with entire forest areas in the vicinity of cement works which are covered by a thick cement crust and have died out. -- Now, we would be interested in knowing what sediment quantities are measured here? In the mentioned region we have found between 1 and 2 g/day/m^2. Thus, these are the amounts which cause, in nature, the damages ascertained by Prof Czaja.

Hesse, Leipzig:

In connection with the detailed transpiration measurements which we have carried out at Leipzig, I am interested in the transpiration process in plants which possess a deposit of lime.

Holte, Bochum:

The concern with the question of the physiological effect of solid, liquid and gaseous substances on plants has led, differential-diagnostically, to the recognition of specific alteration characteristics in the cell- and tissue structure. I can fully confirm, on the basis of my own investigation, the statements made by Prof Czaja regarding the changes in the cell body brought about by cement- and lime dust. In the course of microscopic studies with smoke-affected plants, I was able to establish similar signs of alteration cuticles, epidermis, chloroplasts and cell plasm.

Bohne, Bad Godesberg:

If, through the deposition of dust from cement works, a change takes place in the interior of the plant cell, as demonstrated by the lecturer, then it is understandable why the forests in the vicinity of cement works may die out. The interpretation of the annual rings of these trees -- pine, spruce and poplar -- demonstrates that with increasing production since 1950 there has occurred a very marked decrease of growth (much narrower rings).

Cows grazing in the near vicinity of cement plants have 3 to 5 times more calcium in their feces than non-affected animals. By means of regular rotation, it could be demonstrated that the calcium-content increase had set in already after three days of grazing on the pastures in question.

From the milk-control data, it may be noted that animals that have grazed on meadows lying 1 km to the east or northeast of two cement plants gave in the dry year of 1959 on an average about 1,000 kg more milk than in the three previous years and in 1960. By far the greatest amount of this increased milk quantity was milked during the grazing period. In 1959, the proportion of wind coming from the west and southwest, by which the dust is transported to these meadows, during the period from 1 May to 31 October (grazing season) was very low in terms of the total amount of wind, while in the other years these winds predominated.

Prof Czaja (Concluding Remarks):

I would first like to deal with the remark of Dr Schonbeck of Bochum. Naturally, it is possible for other dusts, for example, coal dust, brown-coal dust, etc., to give off also other substances which act on the cells and produce

phenomena similar to those caused by $Ca(OH)_2$. This must not necessarily be only $Ca(OH)_2$.

Also, I have noted very thick cement crusts. In Sotenich in the Eifel, for example, it is possible even to measure it with the centimeter stick. There, so far as I have been assured, the dust came always from the packing plant in the vicinity of the cement works. But, naturally, that, too, is cement. Now, the firmly bound layer is very thin; its thickness cannot be above 30 μ. This is a property of the cement; this is determined by the cement itself. But on this layer naturally who knows how much more cement may deposit, which naturally conglomerates in the air in a grainy manner. The layer can well amount in thickness to 10 cm. The conditions of crust formation are very complicated. One may scatter a large amount of cement -- powdered, and whenever possible, further pulverized in the mortar -- on a leaf in order to further increase the total surface, but there still will not develop any set crust nor any damage. Just how the layer set on the surface arises in nature when the cloud settles is not known to me in its individual details. Perhaps meteorological factors also play a part. The gentlemen at the Darmstadt Weather Station have established that the cement particles are naturally also condensation nuclei. At the moment, I am engaged in intensive work regarding these questions. It is possible that the transpired water prepared on the leaf surface and a thin hydrate sheath which is brought along by the cement particles, work together and that it is in this way that the set layer comes about. As has been stated, the conditions are not yet clear. When one scatters cement only loosely in an experiment, nothing needs to happen. Thus, it is not yet possible to explain with certainty all phases of the formation of the cement crust as well as its consequences, the injury to the cells.

Now, to the objection of Dr Funke of the cement industry. My investigations -- those that will be published shortly and those which I have briefly communicated in this report -- have been carried out only with dust that had been deposited from the chimney emissions, and it was in this form that they occurred in nature. Thus, this has doubtless been a kiln dust. Despite this, it was an active dust or else it could not have formed a cement crust. The cement-particles must bring along a full readiness for hydration when they fall on the leaf, or else a crust could not develop. Now, as far as the discussion is concerned, I would naturally be very glad to accept the invitation.

Dr Wetzel, Bochum, has mentioned before the enormous amounts of cement dust that may fall per square meter per day, and I must state that all material -- and I have often received materials for investigation -- has behaved, with its two-layered cement crust, exactly as it was presented and reported to you here. I have prepared hundreds of microphotographs in this regard.

Now, Prof Hess of Leipzig asks about the transpiration in the case of a lime deposit. I have not carried out transpiration studies with plants dusted with cement or lime. Besides, I believe that such an experiment with cement dust would a priori be senseless since in these cases there is present an absolutely tight crust which stops cell gas-exchange. In the case of lime, the point of origin of the crust formation is very similar to that of cement.

Dr Holte has essentially confirmed my findings, and so did Dr Bohne. Of quite particular interest were his investigations in regard to the milk production of cows. I must add that, naturally, cement- and lime dust always impresses the mucosa of animals very intensively, since a pH of 12 always represents a quite considerable alkalinity.

Translation No. 863

Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; p 103 - 108

II. GROUP OF LECTURES:

EFFECTS OF AEROSOLS ON MAN

Through Allergizing Aerosols
Through Occupation-Determined Aerosols
Through Disease-Causing Agents

Leader of Discussion: Prof Dr Kliewe, Institute of Hygiene of the University of Mainz (Hygiene-Institut der Universitat Mainz)

Honorary Chairman: Prof Dr L Friberg, Karolinska Institute, Stockholm.

Through Tobacco Smoke
Through Transportation Aerosols

Leader of Discussion: Prof Dr A Goetz, Institute of Technology, Pasadena (USA)

Honorary Chairman: Prof B Tebbens, Sc. D. Industrial Hygiene Engineering, School of Public Health of the University of California, Berkeley

Through Civilization Aerosols
Through Radioactive Aerosols

Leader of Discussion: Prof Dr E Effenberger

Honorary Chairman: Prof Dr H Luther, Institute for Fuel Engineering (Institut fur Brennstofftechnik) of the College of Mining (Bergakademie), Clausthal.

EFFECTS OF ALLERGIZING AEROSOLS ON MAN

AEROSOLS AS ALLERGENS

by Prof Dr K Hansen

Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; pp 104-108

Aerosols or suspended substances are solid or liquid materials (dust, smoke resp. mist) of a particle size between 150 and 0.5 µ dispersed in gaseous dispersion media. Because of their very large surface area they are very surface-active and correspondingly active (depending on their particular quality) on contact with the skin or mucosa. On inhalation, they penetrate, depending on their diameter, deeply into the bronchi; we include here particles whose diameter is below 12 µ. The larger diameters, that is, those over 12 µ, are captured in the upper respiratory passages (oral-, nasal and pharyngeal mucosa); at any event, they become effective at those points. For non-infectious inflammatory diseases of the respiratory tract, Gronemeyer has made a clear and correct differentiation between

1) Direct action through
   a) Chemical
   b) Physical causes.

2) Indirect effects through
   c) Allergical causes.

Today I would like to discuss the indirect aerosol effects on the respiratory tract, that is, the allergical effects.

Allergy

Although the term "allergy" is currently in wide use, there does not exist a clear presentation of the concept

"allergy." In order to differentiate from the direct, immediate reaction of the skin, mucosa and other affected organs brought about by chemical or physical noxae (including the "toxic" poison effect), the allergical reactions develop through an intermediate action induced by the noxa in question, an action consisting in a specific conversion of the organism. Only when this has occurred can the drama unfold. Substances foreign to the body, denoted by the term "allergen," are in themselves harmless, non-poisonous; they call forth, however, when they penetrate into the "internal environment" of the body in an "unprepared" form, a "specific" alteration of the body's reactive capacity in such a way that the person reacts, on repeated invasions of the allergen or allergens in question, with a "shock" whose outcome may sometimes be even fatal. The allergen as such is "neutral"; it acquires, however, a very different, aggressive, yes, life-endangering character when it is able to break through the normally tightly shut skin and mucus-membrane barrier and spread in the body through the blood- and lymph vessels. Normally, the organism absorbs a substance foreign to it only when it has first decomposed it (as in the digestive process) into biologically indifferent molecules or molecule groups, from which it then builds up its "own" body-adapted substance. It responds to the non-physiological influx of substances foreign to the body by the formation of "antibodies," proteins of a globulin character which possess a strictly specific structure (matrix) and reaction ability toward the foreign body in question. For this it needs a period of time of about 9 days; the antibodies are formed in the plasma cells and brought after their maturation into all body cells via the blood- and lymph vessels; the antibodies are then fixed and rested in the cells as "cell-positioned antibodies," These antibodies, too, are <u>per se</u> harmless; they represent a boundary protection which does no harm to anyone who keeps himself outside the "regio sacra" of the individual. Nevertheless, when there is an attempt at a renewed break-in on the part of the substance foreign to the body, the allergen, which the antibodies know and which is the source of their existence, then the antibodies become dangerous. Now, in contact with their allergen, there takes place -- in an explosive manner -- a transformation, the so-called "antigen-antibody reaction," during which the allergen which has penetrated is broken up, "neutralized," under formation of histamine and histamine-like substances. The reaction products of this process, histamine, etc., exert some unpleasant side reactions on certain tissues ("shock tissues"): the smooth musculature and the capillaries. Through these organ effects they cause, in the case of a large overflow, an "anaphylactic, mostly fatal shock"; under certain circumstances only shock fragments. The latter occur when the

antigen-antibody reaction remains limited to the site of attack of the allergen, and takes place there, contact-bound to the skin or the mucosa. This is the rule under the natural exposure- and contact conditions; then the result is a local hyperergic inflammation in the tissue, the reaction of the allergen runs its course at the site of attempted penetration and is thereby prevented from penetrating into the body and there inflame all cell-positioned antibodies. The local reaction is manifested clinically as inflammation of the conjunctiva, as grippe, as bronchitis, even -- as the case may be -- as an attack of bronchial asthma; after a few hours or days, the tissue damage is regenerated and the related functional disturbance, the disease symptom, healed. For the choice of reaction area, the particle size of the corresponding aerosol allergen is of importance: when the diameter is over 12 µ, as for instance in the case of the pollens of gramineae, linden, compositeae, then they only penetrate as far as the pharyngeal mucosa; the reaction process takes place at the upper respiratory passages. The spores of mold fungi are smaller than 12 µ in diameter; they are inhaled in the bronchi and bronchioli; the consequences of the reaction are more dangerous -- bronchial muscle spasm, mucus secretion and swelling of the mucosa -- and easily cause true and severe bronchial-asthma attacks; these reactions are not resolved so fast.

The foregoing is a didactic, relatively simple scheme. I do not speak here of the pathophysiological chain reactions; let it be said only that not infrequently it is possible, when a large volume of particle sizes of aerosol allergens is present which attack only the upper respiratory pathways, that the reactions are transmitted to the deeper passages as well with the formation of bronchial asthma.

However, the precondition for the total allergic disease complex is always that there had taken place a first penetration of the allergen weeks or months earlier, and a sensitization, that is, an antibody formation had occurred. This first and second phase or "preparation" is clinically mute and is often not recognized.

Not counting certain exceptions, the primary attack of an allergen never takes place through a healthy intact mucosa; only when permeability disturbances are present -- whether due to mechanical, chemical, bacterial inflammatory or other injuries or vitamin A deficiency -- is the break-in possible. Exceptions: a) Influx of huge amounts of allergens, and b) particularly potent allergens such as, for instance, the dust of castor beans where the allergen is bound to a

substance with primary inflammatory properties, namely ricin, which serves the allergen in a capacity of pacemaker, etc.

Pollen and spores of mold fungi, of which we have spoken several times, are by no means the sole allergens of the aeroplanktons, the only allergens of organic origin. The number of the latter is very large, but not all of them are dangerous, since they can only occur under given natural environmental conditions in the smallest possible amounts, so that our exposure to them is minimal. It is different with persons who, because of their profession, come into close and frequent contact with them. Dr Gronemeyer will give us individual details on this matter.

In general, substances acting as allergens are of organic origin. They derive from plants, animals, humans, but not exclusively so: inorganic substances, too, can acquire allergenic potency. Still, I will mention, without going into the subject any further, of an in-between region, that is, substances which are supposedly inorganic but which contain an organic substance of which we are not aware or did not detect. Dr Gronemeyer has masterfully analyzed a nice example of such an agent; since he is too modest to bring it up himself, I will so so myself. A supposedly purely infectious disease picture whose cause at first could not even be conjectured; the highly fascinating clinical details are of no interest here. The recidivist attacks with high fever had been caused by inhalation of a metal-purification powder which had been stirred, for purposes of refining and deoxidizing, into a cast-metal bath. Of all components of the powder, the blame had remained attached, in various exposure experiments, to "calcium carbonate," but it could not be understood why this should be so since calcium carbonate is not an allergen. But then it turned out that one had added -- as a plant secret, and therefore not revealed -- oyster shells to the calcium carbonate, as an adsorbent. And <u>this</u> was the allergen, as was definitely proved by skin tests as well as by the antibody test in passive transfer according to Praussnitz-Kunstner. Now this was, as the appropriate tests unequivocally proved, no full antigen, but a haptene, in any event of "organic" origin.

In this connection, I would very much like to know if it is not true that the chronic bronchitis and asthma attacks observed during pearl- and horn polishing are also of an allergic nature and not caused by the physical effects of dust, as has heretofore been assumed.

After this digression, I now come to the "pure" haptenes as aerosol allergens:

It is possible also for protein-free, even inorganic, substances to become allergens under certain conditions and act as such. The entire structure of the science of anaphylaxia and allergy has experienced a decisive extension in 1924 when Landsteiner demonstrated that also substances of relatively simple chemical structure -- for example, diazotized atoxyl -- can acquire antigenic properties. As "full antigens" they only act, to be sure, in a combination with a protein; thus, they sensitize as "azoproteins" and act as antibody-building agents; nevertheless, the protein-free radical alone is able to release the shock in sensitized animals, that is, to bind antibodies. Landsteiner named these bodies half-antigens or haptenes. "Haptene," because the protein as "carrier" serves for its completion into a full antigen. To attain the full antigenic power in the test tube, it is sufficient to mix the haptene with a protein solution; in vivo this carrier function is taken over by the serum protein. This was a highly significant discovery, and only through this do earlier erroneous or completely unexplained clinical observations become understandable. For example, the allergic reaction toward protein-free medicaments administered enterally or parenterally. We know now the allergenic resp. haptene effect of aerosols entering through inhalation -- for example, formalin, acetone and simple aliphatic aldehydes: naphthalene, ursol and other coal-tar dyes (p-phenylenediamine), even of mercury, lead (salts), nickel, etc., as well as the so-called "home-dust" (even when it is free of pollen and fungus spores). To determine how often this occurs and what other organic substances are included in this haptene activity is the great task of industrial medicine and aerosol research. For the aforementioned examples, we have available classical investigations; but how great is the actual, not only the potential, danger? And what conditions aid the onset of this danger?

In this area one has still some serious work to carry out, but we see the road by which it can be accomplished.

I will conclude at this point. Aerosols having allergenic properties such as mentioned above act upon us intermittently, sometimes abundantly and frequently, sometimes sparsely and infrequently. That they attain a pathogenic effect depends to some extent on the constitution of the affected person; to be sure, this is true to a much lesser extent than had previously been supposed. The allergical reaction is a general pathogenic principle, and every person can be sensitized. Hence, the conditions of exposure

seem to me much more important, and among these again those acquired aiding conditions that lead to a penetration of the allergens into the "internal environment" and thereby to a sensitization. Among the factors increasing the permeability of the skin and mucosa, mechanical injuries of the boundary surface play a not inconsiderable role: they produce a lesion of the tissue through which the allergen can freely enter the internal part of the organis; the consequence is sensitization, that is, antibody formation and readiness for shock.

Hence, in our particular interest in the allergen, we should not overlook the importance of the mechanical and chemical injuries caused by non-allergenic dusts, by smoke and mist, that is, the possible factors preparing the road for an allergen attack. This naturally implies the necessity of a joint work in aerosol- and allergy research: such a cooperation would be enhanced by the expectation of notable scientific and practical results.

Translation No. 864

INHALATIVE OCCUPATIONAL ALLERGIES

by Dr W Gronemeyer

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Fortschritte der Biologischen Aerosol-Forschung in den Jahren 1957-1961; pp 109-119

Due to the new regulations issued on 28 Apr 61 regarding the extension of accident insurance to occupational diseases, the inhalative occupational allergies will in the future receive much more attention than has so far been the case. This is so since, according to the 6th occupational-disease ordinance, item 41 on the list, all plants are obliged to pay compensation in the presence of a "bronchial asthma caused by the job which has forced the worker to give up his occupation or all gainful employment." Thus the origin, prevention and treatment of inhalative occupational allergies acquire, beyond the clinical problems involved, a general as well as a social-medical importance.

Under the "inhalative occupational allergies" we understand disease phenomena which

I) are caused by an allergical pathogenesis;
II) lead to the development and/or triggering of disease phenomena as a result of inhalation;
III) are exclusively or predominantly determined by occupation.

We can give here only a basic presentation in regard to the above three points, well aware of the fact that important individual details are omitted.

I. The demonstration of allergy as the determining factor is accomplished through the employment of the critical

criteria of the allergic modes of reaction. These are:

1) The inquiry after a detailed allergy anamnesis;
2) The demonstration of specific antibody production;
3) The demonstration of the presence of the identified antigen;
4) The withdrawal test;
5) The demonstration of therapy, that is, the positive result of treatment by a specific desensitisation with the industrial antigen in question, for example toward flour, insects, dust of exotic woods, etc.

1) Briefly in regard to the taking of anamnestic history, the following points should be particularly kept in mind sub specie an inhalative occupational allergy [1]:

a) The specific conditions at the place of work such as dust evolution, air conditioning, ventilation; these are best found out by a visit to the place of work.

b) Accurate knowledge of the work process.

Thus, a designation such as "silk weaver" is in no way an adequate one, since it does not reveal what work materials are being elaborated, whether raw silk or degummed silk, artificial silk, colored or colorless silk, etc. Thus, for instance, according to the studies of Fuchs [2] the allergic sericin asthma of the raw-silk weaver is observed exclusively in the weavery, brought on by the scraping-off of the gum from threads on running through the reed, and not in other places of work of the spinning works, such as, for example, the places where spooling, winding or threading is done.

c) The method of work is often responsible for the particularly high doses of exposure; for example the employment of a spray gun [3], atomization of working materials through jets, for example in printing plants [4], the massive evolution of dust in grinding plants [5], and so on.

d) The ascertaining of antigens linked to the plant but which are not specific to the occupation (see later).

e) Knowledge of work materials -- not only the finished products but also the intermediate products and, possibly, also the masked admixtures [6], impurities and by-products such as mites, insect larvae, mold fungus spores, etc.

2) In the focal point of the diagnosis stands the detection of the pathogenic antigen or of the formation,

induced by the penetration of the antigen into the organism, of a specific antibody as the indicator of an active sensitization. -- A detection by means of the direct antigen tests on the patient resp. in transfer test according to Prausnitz and Kostner is to be considered as certain proof of an active sensitization. The detection succeeds without any difficulty in the case of all industrial antigens insofar as they are of organic origin, such as is the case of hairdressers' asthma, in the so-called agricultural asthma, in flour asthma, sericin asthma, printers' gum-arabic asthma, in occupational insect asthma, industrial mold-fungus asthma, the so-called carpenters' or wood-sawers' asthma, etc., in other words in the case of the so-called "full antigens." Much more difficult is the antigen detection -- analogously to the situation with the medicinal antigens [7] -- in the case of those antigens which are not "full antigens" but so-called "haptenes" or half-antigens, for instance in the case of ursol asthma of the furrier, the formalin asthma of the painter and anatomical-specimen preparer, etc. Here certain definite variation of the cutaneous diagnosis through conjugation of the antigen to protein in vivo as well as in vitro may be of further help. We shall not here go into any methodological details.

3) For the evaluation of a positive skin reaction the following hint is indispensable: A skin reaction indeed shows -- performed lege artis -- the existence of a sensitization. This can, nevertheless, be clinically mute or subliminal, or its pathogenecity may already have been overcome such as in the case of a survived hayfever. In other words, we may have a latent or apathogenic sensitization or -- as Tuft [8] has described it in more exact terms -- a so-called "non-clinical allergy." Whether, now, the detected antibody actually corresponds to an active sensitization must and can be clarified only through the performance of withdrawal and exposure tests. In this connection our so-called "inhalative antigen-pneumometry test" for the determination of the presence [9] of the antigen has proved its worth in more than 1,000 investigations [10]. I will not here go into individual details of methodology [11]. Regarding this subject as well as the principle of the significance of diagnosis with antigen aerosols I refer you to the lecture of E. Fuchs [12] (see page 357).

How important the clarification of an active sensitization is, particularly in view of the compulsory indemnification for inhalative-occupational allergies, is shown in a table of my collaborator Oehling [13] on so-called asthma of wood workers (see Table I).

TABLE I.

Occupational Asthma of Wood Workers

Positive intrakutane Reaktion bei 67 Arbeitern											
Abachi	Ahorn	Buche	Eiche	Limba	Mahagoni	Makoré	Mansonia	Nußbaum	Palissander	Ramin	Teak
10	1	—	15	7	3	13	11	5	6	13	16
Aktuelle Sensibilisierung: 12											
5	—	—	1	2	—	3	3	—	—	—	2

1 -- Positive Intracutaneous Reaction in 67 Workers; 2 -- Abaca; 3 -- Maple; 4 -- Beech; 5 -- Oak; 6 -- Limba; 7 -- Mahogony; 8 -- Makore; 9 -- Mansonia; 10 -- Walnut; 11 -- Rosewood; 12 -- Ramin; 13 -- Teak; 14 -- Active Sensitization.

4) I will not go further into the subject of the withdrawal -- as well as the natural resp. re-exposure test at the place of work. Their significance and operation in the case of inhalative occupational allergies are self-evident.

5) As the last criterion of the allergic mode of reaction I named the positive result of treatment after the performance of a specific desensitization [13, 14]. I would like to emphasize that the inhalative antigen pneumometry test has proved its worth also in the control of the course of a desensitization [15].

II. The route of invasion of an antigen is of twofold importance for the clinical symptom analysis: in the choice of organ of the allergic reaction and the group classification of the suspected and sought antigen.

Thus, corresponding to the contact rule formulated by Hansen [16] the clinical picture of the inhalative occupational allergies includes, first of all, preferentially, hyperergic reactions on the mucosa of the upper and lower respiratory passages with their known clinical symptomatology: rhinitis, sinusitis, laryngitis, thracheitis, bronchitis, as well as asthma and related disturbances. To this is to be added -- since its course also runs according to the contact rule -- the allergic conjunctivitis. By these entities, however, the clinical forms of inhalative occupational allergies are by no means exhausted, a fact that is often left out of consideration. Through resorption of

the inhalation antigen there develop, as a result of a hematogenic antigen scattering, so-called distant symptoms, particularly Quincke edema, urticaria and eczema. Thus for example Waldbott [17] was able to detect an inhaled antigen in 35 out of 158 cases of often difficultly analyzable chronic urticaria, among a whole series of industrial antigens such as flour, animal hair, kapok, perfume, mold-fungus spores, etc. Castberg and Sorensen [18] found in their allergy studies in 130 bakers 7 instances of an inhalative flour urticaria. Storck [19] has only recently called the attention to the inhalative release of eczemas, particularly also on sensitization toward known occupational antigens such as terpentine (so-called vapor- or scent-allergy), various antibiotics, mercury, etc.

Insofar as the antigen catalog of inhalative occupational allergies is concerned, this is so extensive that a full listing must be omitted. The same holds true for the different occupational groups and industrial branches. I would like to refer to the 15 occupational categories formulated by Hansen [20] in which inhalative occupational allergies preferentially occur. Because of the constant change and modification of the work methods and the use of new work tools and materials, constant additions, completions and strikings are necessary. This may have been the motive of the legislators not to tie the obligation to pay compensation for industrial forms of asthma to the action of any specific catalog items or antigens but to choose a comprehensive formulation that is not fixed with respect to the pathogenesis of industrial forms of asthma. This is correct, since concepts such as "asthma of flour workers" are in no way uniform etiologically. Not infrequently the sensitization is oriented toward obligatory or optional admixtures or impurities. Thus it is possible -- if only more rarely -- for bakers' asthma to be based on an isolated allergy to flour mites, kitchen scrapings or even the spores of black rust. In the case of asthma of feather workers the sensitization can be directed -- apart from feather dust -- toward mites, moths as well as various mold-fungus types, and likewise in the case of asthma of hairdressers. This circumstance is not only of importance for diagnosis but requires particular attention also in the case of a change of occupation in the sense of the exclusion of antigen as well as in the legal evaluation of the exclusivity of the antigen, a requirement rightly demanded for instance, by Carrie [21] for the allergic occupational dermatoses.

III. And now to the last point, the occupationally determined character of inhalative allergies. For the assumption of an inhalative occupational allergy an additional fundamental requirement besides the demonstration of a current sensitization to the antigenic work substance in question must be satisfied: In contrast to a "spontaneous allergy," in the case of an inhalative occupational allergy those particular states must be demonstrated which are determined by the peculiarity of the industrial activity, namely: the massive exposure and the great aggressivity of the occupational antigen. According to experience the two circumstances often occur contemporaneously, so that one can rightly speak in inhalative occupational allergy -- in contrast to spontaneous allergy -- of a "forced-up" sensitization [22]. Here are some examples and figures:

The printers working with the wet-spraying process are exposed to a particularly intensive inhalation of gum-arabic aerosol. While, in a printing plant -- according to comparative dust measurements of Albrecht [23] -- the number of dust particles increases due to the movement of the paper from 2,400 to 24,000 per liter in the course of an eight-hour working period, during the employment of the liquid atomizer 500,000 to 600,000 dust particles are ascertained already in the first half hour after the beginning of work. As a result of this particularly massive exposure a more than 50% sesnsitization rate among the printers is hardly surprising [24]. As a further example of the exposure factor I cite Schwartz's investigations [25] on bakers: He was able to establish that in small plants with poor ventilation, cramped space, lack of machinery such as, for example, dough-mixing machines and sack-beating machines, the sensitization rate of bakers amounted to 44%, while the figure in larger plants was only 25%. Unfortunately such systematic dust measurements are lacking for many branches of industry of larger or smaller occupational categories. I would mention here the exposure to dust in coffee loading, roasting and sorting [25], in the cutting rooms of large carpentry establishments [13], in the jute-, hemp- and sisal-industry, in the cotton industry (particularly in the combing rooms), further, in the agricultural dust environment (threshing dust, hay dust, etc.).

Not infrequently the highly massive exposure goes hand in hand with a particularly high aggressivity of the industrial antigen. The aggressivity of an industrial antigen is expressed first of all by an often unusually high degree of sensitization. Thus we noted a short time ago in every

member of a biological research team working with African migratory locust in connection with the testing of new contact insecticides a degree of sensitization which could be detected in skin titration up to a dilution of 1:10 billion [27]. The antigenic nature of the skin reaction was established by means of the Prausnitz-Küstner test and the inhalative antigen-pneumonetry test. Grades of sensitization as high as this are in no way rare; they occur in a series of other known occupational antigens. I would mention the allergy to castor-bean dust which, as an occupational asthma, develops not only in the vicinity of ricinus mills or in persons who use or elaborate the cake as fertilizer, but which attacks, as a "collective" and "endemic" asthma, in function of the wind direction, the population within a radius of 3 to 5 kilometers from the plant, as we know from the studies of Ordman [28], Mendles and Cintra [27], Hansen [30], Panzani [31] et al.

However, the aggressivity of an antigen is expressed not only by the degree of sensitization but also -- and to me this seems even more important for the assumption of an inhalative occupational allergy -- the so-called index of sensitization. By this we mean the number of sensitized persons in percent of the total number exposed. In the silkworm industry this index stands at about 25%, for bakers between 26 and 44% depending on the working conditions, for printers 30 to 50%, for occupational insect allergies over 50%. However, here, too, it is to be regretted that systematic plant studies are not available for many industrial antigens, studies which would, in combination with the general exposure conditions, give some information regarding the potency of the antigen as well as the relationship of mute to manifest sensitization; data which ought to form the general bases and prerequisites in the light of the compulsory indemnification for inhalative occupational allergies. The difficulties which stand in the way of such investigations are of several types. I will not, however, go into them any further.

In conclusion let me mention a few viewpoints which are of general significance for the examination and evaluation of insurance claims. In regard to the question of whether the causative antigen is present solely or predominantly during the work or encounters the patient also outside his occupation, the following categories may be set up according to Spain and Fontana [32]:

a) The encounter with the antigen is fully and exclusively limited to the place of work. Extra-occupational contact possibilities are as good as absent. Example: printer's asthma.

b) There exists to a far lesser extent than the massive exposure at the place of work, a more or less limited contact with the antigen outside the occupational environment. Example: Feather-occupational asthma in a female worker in a bedding factory with massive exposure to dust as compared with the relatively slight contact with feather at home.

c) The occupational antigen is very abundantly present both at the place of work and in the extra-occupational milieu. Example: Street cleaners or rag pickers with a mold-fungus allergy; vacuum-cleaner salesmen with an allergy to home dust, etc.

d) There is present an extra-occupational sensitization which induces the onset of complaints through unspecific additional irritation at the place of work such as dust, smoke, smell, etc. This is the case -- as we have termed it -- of a "secondary susceptibility asthma" [33]. In other words, it is a question of an apparent exposure and no true inhalational allergy. For the acceptance of an indemnity obligation situations a) and d) present, in my experience, no difficulties, while b) and c) do quite a bit; however, I shall not go into this any further.

I have attempted to present a few of the general aspects of the development and evaluation of inhalational occupational allergies. Certain facets of the multilayered problem have only been raised and knowingly not discussed, such as, for example, the primary or secondary significance of grafted infection, the constitutional question, the extent of psychic factors, etc. These factors which may also be considered formative factors are able to determine the course of the individual disease from case to case; in the case of inhalative occupational allergies the peculiar and primary disease potential always grows out of the special, occupation-determined magnitude of exposure in combination with the particularly high aggressivity of the industrial antigen.

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Discussion

Symanski, Saarbrucken:

Dr Gronemeyer has spoken about the so-called printers asthma. The impression arose that about 25-30% of all printers have become ill with this printers' asthma which is to be attributed to inhalation of gum arabic. But as a matter of fact today instead of gum arabic, lime dust is used in the printing plants.

I would like to direct a second question to Prof Hansen. He spoke about the fact that certain substances act as haptenes, which in combination with albuminates may act in certain circumstances in an allergy-producing manner. We have, however, never been able to observe in industrial-medical practice allergic phenomena after inhalation of the finest lead dust. Nor can I remember ever having read in the literature about lead allergens.

Groetschel, Wiesbaden:

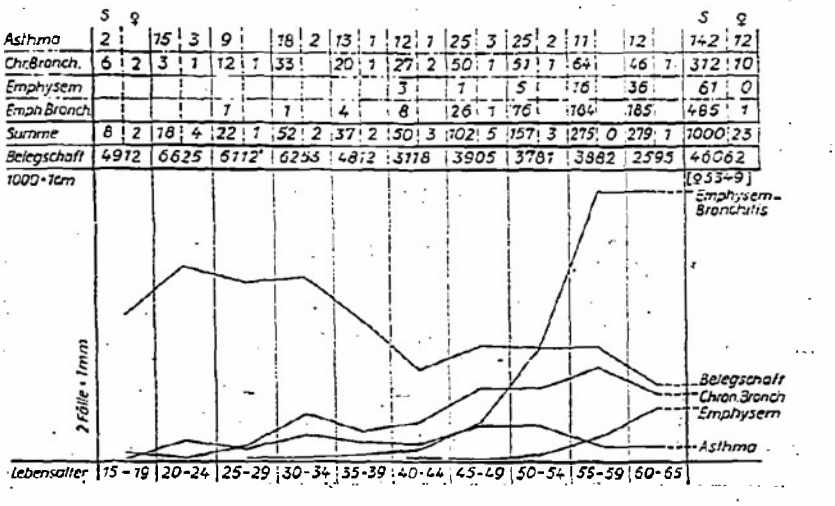
So far I have come across only a single case of lead asthma in the literature. The allegation is contained in the book, "Allergic Diathesis and Allergic Diseases" by Hugo Kammerer and Hermann Michel, 3rd Edition, J. F. Bergmann Publisher, Munich (1956), pp 375m 664 and 685.

Hilgenfeld, Ludwigshafen:

In regard to the question of Prof Hansen: "How often does it actually occur?" I would like to show you a numerical table from a large chemical plant which may, to a certain extent, be considered representative. All disease pictures of respiratory insufficience were impartially counted in 46,000 persons in the various age groups; these persons were all workers at the plant. The bottom row of figures indicates the number of workers in each age group. The upper curve, decreasing from left to right, likewise represents the number of personnel in each age group.

The upper row of figures comprises the symptomatology of asthma in the form in which the general practitional conceives the clinical asthma, that is, without constriction of the vital capacity, without bronchitic rattles in the interval, but with classical asthma attacks. The corresponding curve is denoted in the graphical part by the term "Asthma." It was noted that, first, the number of asthmatics does not

increase with increasing age, and second, the fluctuations run parallel throughout to the number of persons in the individual age groups. The sexual distribution of clinical asthma (12 women out of a total of 142 asthmatics) too, is parallel to the ratio of 5,300 women out of a total personnel of 46,000.



1 -- Asthma; 2 -- Chronic Bronchitis; 3 -- Emphysema; 4 -- Emphys. Bronchitis; 5 -- Total; 6 -- Number of Persons; [S = Total]; 7 -- 2 cases = 1 mm; 8 -- Age; 9 -- Emphysemal-Bronchitis; 10 -- Number of Persons; 11 -- Chronic Bronchitis; 12 -- Emphysema; 13 -- Asthma.

The second curve from the bottom corresponds to emphysema, clinically characterized by the decrease of the respiratory volume without catarrhal phenomena and attacks. It only acquires a significance after the 50th year.

The third curve describes the symptomatology of chronic bronchitis characterized by no noteworthy reduction of the respiratory volume, but with wet noises. The curve begins already at the 15th to 19th year, and then rises slowly but steadily with age.

Most striking, however, is the curve for the emphysemal bronchitis. It rises sharply from the 40-45th year on, and so much in fact that already with the 60th year a morbidity of 10% of the personnel is to be observed. By

emphysemal bronchitis we mean the disease entity which proceeds without characteristic attacks of shortness of breath, without eosinophilia, but with marked constriction of the vital capacity and with catarrhal phenomena. The reason for the abrupt increase of the morbidity is as yet entirely unclear, and is being exhaustively investigated through exact work histories (anamneses) in each and every case.

Hansen, Heidelberg (Concluding Remarks):

Mr Symanski: I did not speak of lead asthma, only about lead as one among the numerous substances with haptene effect. I personally do not know of a lead asthma; but do know very well, for example, mercury asthma. An allergy to lead salts as contact allergens has been described. Likewise to nickel and cobalt. All these substances act not only as "poisons" but also as haptenes or half allergens. And it was for this reason that I pointed it out, since it would be so necessary that all industrial personnel working with these inorganic antigens be tested for a facultative or latent sensitization. The question involved is the possibility of detecting persons in the sensitization stage in good time -- that is, before the manifest illness -- and then taking them out of the plant already at that time.

Mr. Hilgenfeld: We have already conversed so often, and I am always learning something new from your words. The curves which you have shown today do not, however, tell me anything new about the pathogenesis of the employees. "Constitutional," "essential?" Yes, perhaps. If one were, however, to investigate systematically the cause of the accompanying or prior bronchitis, then one would (after all other experiences with asthma through inhalation of occupational dusts) probably find a number of sensitizations also in these "emphysemal-bronchitic" patients. These persons could then be protected from future exposure to antigen and from a deterioration of their emphysema as well as premature invalidism.

Gronemeyer, Bad Lippspringe (Concluding Remarks):

To Prof Symanski: Our own investigations were carried out two years ago in a large printing plant in North Germany, in which at that time some 20 printers still worked with the wet-spray process. It appears to us essential that it is not only the printer working directly at the machine that is exposed but also -- as is rightly emphasized by Albrecht in his cited paper -- the atmosphere of the entire working

space, and therefore all those working in the room are exposed. In 1955, as far as I know, W. von Hoscheit established that in Heidelberg there were still about 3,000 machines with wet spraying in operation.

To Dr Hilgenfeld: I may perhaps say yet a few words: From his curves we have been able to note how often in the heavy chemical industry one encounters pulmonary insufficiency phenomena amounting to an illness. The more important question however, that interests us in connection with our subject should not be, as I have shown, "how often do symptoms occur," but "where and at which place of work do symptoms occur?" a differentiation which is not evident from the curves of Dr Hilgenfeld. Thus a total-statistical evaluation such as is presented by Dr Hilgenfeld can hardly, if at all, help us further in the clarification of etiological relationships.

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